Pentoxifylline in anaemia resistant to erythropoietin (PEAR) study

A double blind placebo controlled randomised trial

Thesis submitted for the degree
Doctor of Medicine (Research)

Queen Mary University, London

Tarun Kaushik

Translational Medicine and Therapeutics

William Harvey Research Institute

Queen Mary University, London

United Kingdom

Declaration and Acknowledgements

The work presented in this thesis is my own (unless stated). It was conducted in the Department of Nephrology, The Royal London Hospital, London, UK .

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Dr Tarun Kaushik

MBBS, MRCP Nephrology (UK)

Sept 2018

Statement of originality

I, Tarun Kaushik, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

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Date: 26. September. 2018

Details of publications:

November 2015: Poster presentation at American Society of Nephrology meeting "Incidental Findings on 15 Fluorodeoxyglucose Positron Emission Tomography Along with Low Dose Computerized Tomography (FDG PET CT) Scans Among Clinically Stable Haemodialysis with Erythropoietin Stimulating Agent (ESA) Hypo Responsiveness (ESA-R)"
T Kaushik, S Fan, M Yaqoob

Abstract

Background

Hyporesponsiveness to erythropoiesis-stimulating agents (ESA) and its association with adverse cardiovascular outcomes remains a considerable problem in patients with end-stage renal disease (ESRD) undergoing haemodialysis. Pentoxifylline has been shown to have some beneficial effect on ESA Hyporesponsiveness by reducing inflammation in ESRD patients.

Methods

We conducted a single centre, double-blind, placebo-controlled randomised trial to study the effect of Pentoxifylline on Erythropoietin stimulating agent (ESA) requirement of stable haemodialysis patients. Inclusion criteria were equivalent ESA dose of greater than or equal to 6000 International Units (I.U) per week or ESA resistance index greater than or equal to 6.5 I.U /kg/wk/Hb (g/dl) and stable Hb between 9 to 12 g/dl. The primary study endpoint was ESA requirement relative to Haemoglobin (Hb) level at the end of study period of 6 months. Secondary endpoints included safety analysis, Hb values, ESA dose and cardiovascular imaging biomarkers such as vascular PET CT and cardiac MRI scan. Cytokine profile was also analysed during the study.

Results

A total of 69 patients underwent randomisation. At the end of the study period, there was no statistically significant (p value= 0.26) difference in ESA /Hb ratio between pentoxifylline and placebo group (Mean (SD) 3.98 mcg/gm/dl (3.09) versus 4.91 mcg/gm/dl (3.49) respectively). The secondary outcomes did not show any statistically significant change between pentoxifylline and placebo group. There were no concerns regarding the safety of pentoxifylline in haemodialysis patients. The cytokine profile showed a reduction in inflammatory cytokines titres and rise in anti-inflammatory cytokines in the entoxifylline group analysed as slopes of cytokine variability longitudinally.

Conclusions

Pentoxifylline did not improve the ESA requirement in ESA hyporesponsive, stable haemodialysis patients over six months period. There was no statistically significant change in cardiovascular imaging biomarkers between Pentoxifylline

and placebo group. Cytokine profile showed a favourable response to Pentoxifylline therapy.

List of abbreviations

Abbreviation	Meaning
ACEinh	Angiotensin converting enzyme inhibitor
ARB	Angiotensin receptor blocker
AD	Aortic distensibility
AE	Adverse events
Ao	Aorta
ATP	Adenosine triphosphatase
Akt	Protein Kinase B
AVF	Arteriovenous fistula
B.C.	Before Christ
BFU-E	Erythroid burst forming units
Bcl- xL	B-cell lymphoma-extra
BMP 6	Bone morphogenetic protein 6
cAMP	cyclic adenosine-3,5-monophosphate
C.E.	Common Era
CFU-E	Erythroid colony forming units
CKD	Chronic kidney disease
CMR	Cardiac magnetic resonance
CREB	cAMP - response element binding protein
CRF	Case report form
CRP	C- reactive protein
CT	Computed tomography scan
CVC	Central vascular catheter
cAMP	Cyclic adenosine 3,5-monophosphate
DMT	Divalent metal transporter
DNA	Deoxyribonucleic acid
dl	Decilitres
EKLF	Erythroid Kruppel like factor
EPO	Erythropoeitin
EPO-R	EPO receptor
ELK-1	ETS domain-containing protein gene-1
ERK	Extracellular signal regulated
ERFE	Erythroferrone
ESA	Erythropoeitin stimulating agents
ESRD	End stage renal disease
18 FDG	18 Fluorodeoxyglucose
FIH-1	Factor inhibiting HIF-1
FOG - 1	Friend of GATA 1
GATA 1& 2	Transcription factors with ability to bind GATA sequence of DNA
GDF 15	Growth differentiating factor 15
GM-CSF	Granulocyte macrophage colony stimulating factor
gm	Grams
GN	Glomerulonephritis
GRB	Growth factor receptor-bound protein
GLUT	Glucose transporter

Hb	Haemoglobin
HIF-1	Hypoxia inducible factor 1
HIF 2	Hypoxia inducible factor 2
HJV	Hemojuvelin
HNF 4	Hepatocyte nuclear factor 4
hs-CRP	Highly sensitive C reactive protein
IFN-γ	Interferon gamma
IFN gamma	Interferon gamma
IL-3	Interleukin 3
IL-6	Interleukin 6
ITT	Intention to treat
I.V.	Intravenous
IMP	Investigational medicinal product
ITT	Intention to treat
JAK 2	Janus Kinase 2
Kg	Kilogram
kDa	Kilodalton
kt/v	Dialyser clearance of urea X time on dialysis / volume of distribution of urea
LDL	Low density lipoprotein particles
LM	Left main artery
LV	Left ventricle
LVH	Left ventricular hypertrophy
LVEDV	Left ventricular end diastolic volume
LVESV	Left ventricular end systolic volume
LVEF	Left ventricular ejection fraction
LVSV	Left ventricular systolic volume
MAPK	Mitogen activated protein kinase
MBq	Megabecquerel
mg	Milligram
mm	Millimeter
MMPs	Matrix metalloproteinases
ml	Millilitres
mSv	Millisievert
NO	Nitric oxide
NF-KB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ns	P value not siginifcant
OD	Omne in die or once daily
PET	Positron emission tomography
pg	Picograms
pH	Pouvoir hydrogène (power of hydrogen)
PHD 2	Prolyl hydroxylase domain 2
p-IgA 1	Polymeric immunoglobulin A 1
PI-3K	Phosphoinositide 3-Kinase
PKA	Phosphokinase A
PWV	Pulse wave velocity
RAAS	Renin-angiotensin aldosterone system
RBC	Red Blood cells
RCT	Randomised controlled trial

RHEX	Regulator of human erythroid cell expansion
ROI	Region of interest
SAE	Serious adverse event
S.C.	Subcutaneous
SCF	Stem cell factor
SD	Standard deviation
SHC	Src homology 2 domain containing transforming protein 1
SOS	Son of Sevenless homolog
SMAD	Signal transducer proteins fir receptors TGF beta
SMC	Smooth muscle cells
Spi	Serine protease inhibitor
SSFP	Steady state free precision
STAT	Signal Transducer and Activator proteins
SUV	Standardised uptake value
SUVmax	Maximum standardised uptake value
SVC	Superior vena cava
TAC	Total arterial compliance
Tal-1/SCL	T-cell leukaemia 1 / stem cell leukaemia
TBR	Target to background ratio
TFR 1	Transferrin receptor 1
TFR 2	Transferrin receptor 2
TMPRSS6	Transmembrane protease, serine 6; matriptase-2
TNF-α	Tissue necrosis factor alfa
TGF	Transforming growth factor
THL	Tunnelled haemodialysis line
TIN	Tubulo interstitial nephritis
μm	Micrometer
μg	Microgram
URR	Urea reduction ratio
VCAM-1	Vascular cell adhesion molecule 1
VHL	Von Hippel- Lindau (VHL) protein
VEGF	Vascular endothelial growth factor
Wk	Week
WMD	Weighted mean difference

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1. Introduction

Blood has always been a source of great fascination in human history. Over the centuries, it has occupied minds of scientists, philosophers, scribes and artists. The importance of blood in the preservation of life appears to have been recognised as early as Paleolithic times. Perhaps the circulation of blood was first mentioned in the ancient Chinese and Indian medical literature. One of the Hippocratic writings from approximately 400 B.C. describe blood as being a part of four 'humors' which form the composition of the human body. Subsequently, there were significant contributions by Roman scientist Galen (born 169 C.E), Iranian scientists Rhazes and Haly Abbas around 10TH century C.E. towards increased knowledge of circulation, which formed the foundation of modern theories on circulation[1-4].

In modern medical literature, the discovery of the blood circulation by English Physician William Harvey (1578-1657 C.E.) is regarded as a turning point in the history of modern medicine. The cellular composition of blood was first recognised by a Dutch microscopist Leeuwenhoek who was able to observe red blood cells under a microscope and described size and shape of red corpuscles in an illustration in 1695. The discovery of white blood cells and platelets followed after microscope lenses were improved. Karl Vierordt in 1852 reported the first quantitative results of blood cell analysis[5, 6].
The growth of knowledge of the physiology of blood and improved methods of blood examinations allowed anaemia and other blood related disorders to be studied on a rational basis. In 1900, the discovery of blood groups by Dr Landsteiner paved the way for successful crossmatched blood transfusion. The subsequent improvements in techniques of blood transfusion have made it a safe treatment for anaemia. However, this treatment was not without complications[7].

1.1 Red Blood cells

The four main components of human blood are plasma, red blood cells (RBC) or erythrocytes, white blood cells (WBC) and platelets. Human RBC is biconcave or

discocyte shaped. The discocyte shape of human RBCs is approximately 7.5 to 8.7 μm in diameter and 1.7 to 2.2 μm in thickness. Haemoglobin [8] molecules, essential for gas transport within the circulation, are contained in the RBC cytosol [9]. Haemoglobin is an iron-containing oxygen transport metalloprotein and forms 96% of the red cell content by dry weight.

Haemoglobin [8] a 64.4 kiloDalton metalloprotein tetramer consisting of 4 subunits: two pairs of globin polypeptide chains - a pair of alpha-like chains and a pair of non-alpha-like chains. Each subunit consists of a haem protein chain tightly associated through covalent bonding with a non-protein haem group. The haem group consists of a single molecule of protoporphyrin coordinately bound to a single ferrous (Fe^{2+}) ion, which carries oxygen and other gases.

Red blood cells enable transportation of sufficient oxygen from between lungs and metabolising tissues through their high intracellular content of Hb [8] and allosteric interaction between ligand (oxygen, carbon dioxide and Hydrogen ion) binding sites in Hb molecule. The tetrameric Hb molecule is in equilibrium between two quaternary structures, relaxed 'R' structure with high oxygen affinity and the tense 'T' structure with low oxygen affinity. The oxygen-transporting properties of RBCs show considerable plasticity and can be adjusted to variable tissue O_2 needs and environmental constraints *via* changes in intra erythrocytic pH and organic phosphates.

Red blood cells also contribute to the phenomenon of hypoxic vasodilatation when passing through microcirculation ensuring fast matching of local oxygen supply and demand. The RBCs can regulate their own distribution in the microcirculation through mechanisms such as deoxygenation dependent release of adenosine triphosphatase (ATP) from RBC, which stimulates production of nitric oxide (NO) and other vasodilators in the endothelium; release of vasoactive NO from S-nitroso-Hb upon deoxygenation; and reduction of naturally occurring nitrite to vasoactive NO by deoxygenated Hb [10].

1.2 Anaemia

Anaemia is defined as an absolute reduction in the number of circulating red blood cells. For practical purposes, anaemia is defined as the reduction in haemoglobin, haematocrit or red blood cell count (usually measured as millions of RBCs per microliters). World health organisation definition of anaemia for men and women are haemoglobin less than 13 gm/dl and less than 12 gm/dl respectively. This definition has limitations particularly in a patient population with chronic diseases affecting erythropoiesis.

Causes of anaemia broadly classified into a) abnormal RBC production b) increased destruction of circulating mature RBCs c) blood loss.
In the absence of nutritional deficiencies, genetic RBC disorders and bone marrow disorders, anaemia in chronic kidney diseases occurs as a result of decreased effective red cells production. Hence anaemia in chronic kidney disease could occur as a result of relative or absolute erythropoietin deficiency and inflammation.

Treatment options for anaemia in CKD are as follow:

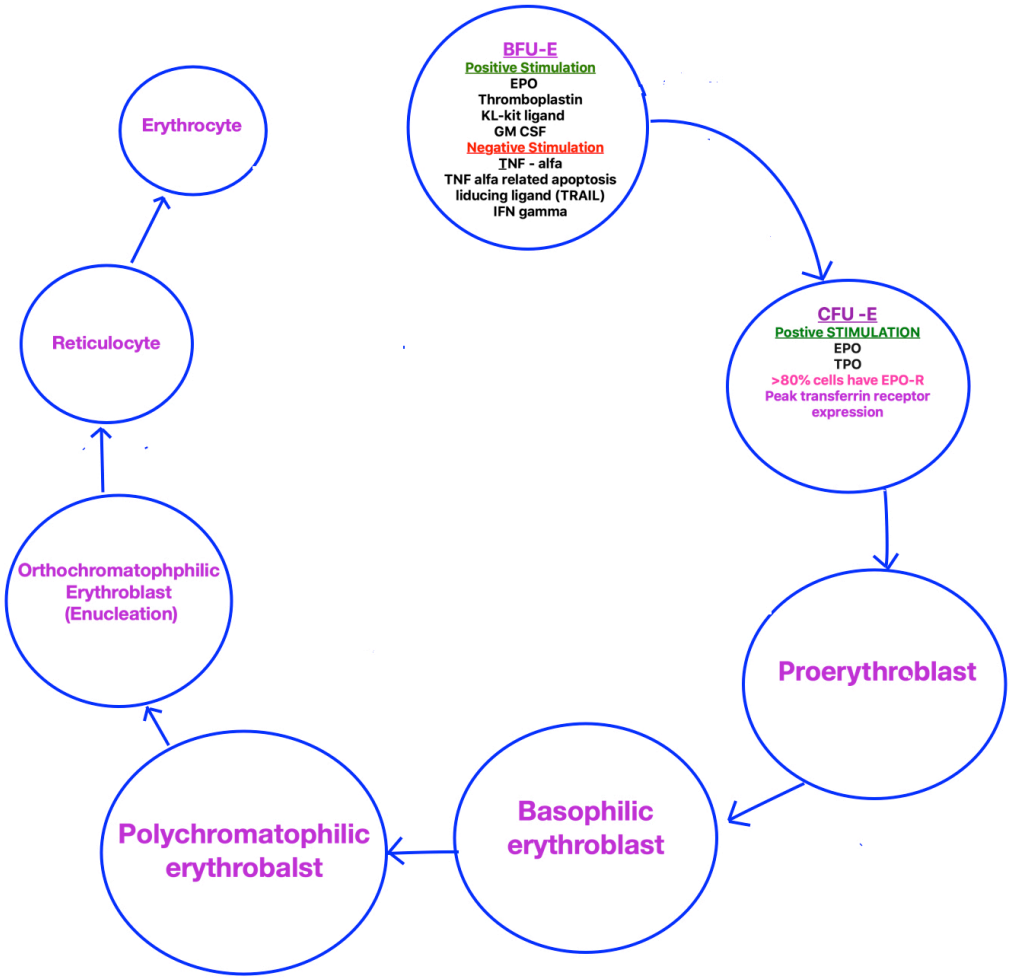
1. Frequent blood transfusions: Although blood transfusion is an effective way of treating anaemia. However, complications associated with recurrent blood transfusions such as iron overload, immunologic sensitization, volume overload in non-dialysis patients and transfusion associated infections are undesirable.
2. Androgens: Androgens by their effect on increased endogenous EPO production, increased sensitivity of erythroid progenitor cells to EPO in bone marrow were the mainstay of treatment of anaemia associated with CKD before the discovery of ESAs. However, side effects associated with androgen limited their use.
3. ESA: As mentioned earlier ESAs revolutionised the treatment of anaemia in CKD patients. ESA eliminated the need for blood transfusion in the majority of CKD patients.

2 Erythropoiesis

Erythropoiesis (from 'erythro' meaning "red" and 'poiesis' meaning "to make" in the Greek language) is the process of producing red blood cells or erythrocytes. The mature red cell is the final phase of a complex but orderly series of genetic events that initiates when a multipotent stem cell commits to the erythroid program. During steady-state haematopoiesis, adequate number of red blood cells are produced per hour in the bone marrow to maintain the haemoglobin level within reasonably narrow limits. The RBC production is rapidly increased in the setting of ongoing blood loss or hemolysis. This process happens in bone marrow, which provides ideal microenvironment consisting of stromal cells, haematopoietic accessory cells and extracellular matrix.

The erythroid progenitor cell compartment is situated functionally between the multipotent stem cells and the morphologically distinguishable erythroid cells, contains a spectrum of cells with a parent to progeny relationship, all committed to erythroid differentiation. This process is regulated by transcription factors and growth factors acting at various stages of development of an erythrocyte from a multipotent stem cell. (Figure 2.1)1

Figure 2.1. Development of an erythrocyte from a multipotent stem cell



The transcription factors, which act at the level of hematopoietic stem cells, include T-cell leukaemia 1 / stem cell leukaemia (Tal-1/SCL) and GATA2, while GATA1, friend of GATA 1 (FOG-1) and Erythroid Kruppel like factor (EKLF) are more critical for erythropoiesis. The growth factors play an essential role in regulating the proliferation and maturation of erythroid progenitor cells. Growth factors include erythropoietin (EPO), stem cell factor (SCF), insulin and insulin like growth factors, transforming growth factor (TGF) beta gene family, interleukin 3 (IL-3) and granulocyte macrophage colony stimulating factor (GM-CSF).

Erythroid burst forming units (BFU-E) are the most primitive cell lineage committed erythroid progenitors, which respond to EPO. The BFU-E arise from multipotent stem cells. Under the influence of EPO and other growth factors, BFU-E forms subpopulations of erythroid colony forming units (CFU-E). Greater than 80% of cells in CFU E population have EPO receptors, indicative of the role of EPO in early stages of erythrocyte development. Transferrin receptor expression is also at peak at this stage as iron is essential for heme synthesis.

The earliest recognisable erythroid cell is pro-erythroblast, which after four to five mitotic divisions and following morphologic changes, gives rise to mature erythroid cells. Its progeny includes basophilic erythroblasts followed by polychromatophilic erythroblasts and orthochromatic erythroblasts. Their morphologic characteristics reflect the accumulation of erythroid specific proteins (i.e. Haemoglobin) and a decline in nuclear activity.

After the last mitotic division, the inactive dense nucleus of the erythroblast is expelled and ingested by macrophages, and the resulting enucleated cell is a reticulocyte. There is a maturation-associated decline in the number of EPO receptors, which parallels the declining influence of EPO on erythroid cells during the terminal phase of maturation. Reticulocytes do not show detectable binding to EPO [11]. Erythropoiesis is regulated by a complex sequence of growth factors such as erythropoietin and cytokines.

2.1 Erythropoietin

Erythropoietin (EPO) is a 35-kd glycoprotein [12], which is a physiologically essential growth factor for red cell production [13]. It is a true hormone as it is mainly produced in peritubular cells in the kidneys in adult life, transported through the bloodstream and then acts in the bone marrow [14]. EPO is responsible for both maintaining normal erythropoiesis and increasing red cell production in response to intracellular oxygen needs.

EPO stimulation elicits two types of measurable responses: changes in the proliferative activity including improved survival and changes in maturation

rates of CFU-E and erythroid precursors which are extremely sensitive to EPO. Virtually all these cells are already in the cycle. Therefore, rise of their numbers cannot be achieved by increasing their fraction in the cycle. Hence enhanced survival of CFU-E could be linked to the crucial role of EPO in terminal differentiation of erythroid progenitors.

In studies done on mice with homozygous null mutations for EPO or EPO receptor (EPO-R) genes, the BFU-E and CFU-E colonies fail to differentiate into mature erythrocytes[15]. In vitro studies have shown that EPO and other hematopoietic growth factors act synergistically to enable terminal maturation of erythrocyte progenitor cells by suppression of apoptosis [16-18]. EPO plays an evolutionarily conserved role in promoting, survival and appropriate timing of terminal maturation of primitive erythroid precursors [19].

2.1 EPO expression

All nucleated cells in the body sense & respond to hypoxia. The rate of oxygen delivery and the level of oxyhaemoglobin remain the fundamental regulator of erythropoiesis. Under hypoxic conditions, hypoxia-inducible factor 1 (HIF-1) [20] regulates expression of genes that mediate adaptive response. HIF-1 acts as a regulator of EPO, vascular endothelial growth factor (VEGF) and glycolytic **enzyme**. HIF-1 may play a role in the regulation of more than 2 per cent of all human genes [21].

The interstitial cells in the inner cortex and outer medulla of the kidney respond to hypoxia by producing erythropoietin. Hypoxia activates production of HIF-1 in these specialised cells, which in turn leads to activation of EPO gene expression following carefully choreographed sequence of events. HIF 1 is composed of a constitutively expressed HIF 1 β subunit and oxygen regulated HIF 1 α subunit or HIF 2 α which has a similar role as HIF 1 α. HIF 1 α is only detectable under hypoxic conditions [20, 22]. Figure 2.2 describes the mechanism of HIF-1 control and activation.

Hepatocyte nuclear factor 4 or HNF 4 also plays a critical role in hypoxia induced activation of EPO gene expression[23]. The Von Hippel-Lindau (VHL)-HIF pathway also mobilises iron to support erythropoiesis by down regulation of hepcidin production and up regulation of ferroportin, facilitating iron absorption and mobilisation [24]

Figure 2.2. EPO regulation through HIF pathway

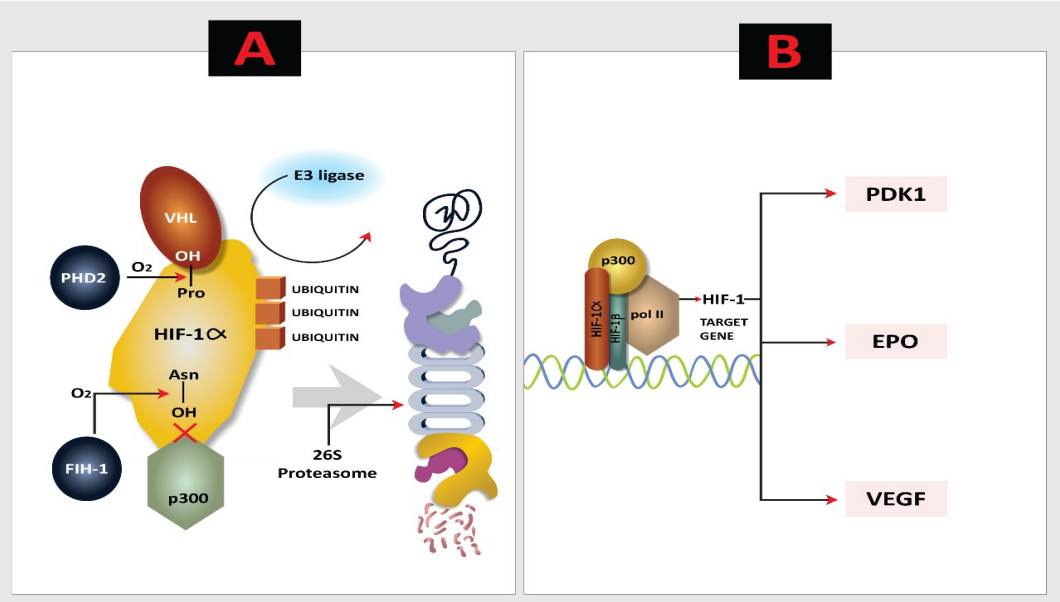


Figure 2.2

In well-oxygenated cells, HIF 1 α is ubiquinated and degraded by proteasomes as shown in figure 2 A. In mammalian cells, the oxygen sensing propyl hydroxylase domain 2 (PHD 2) uses oxygen to hydroxylate HIF-1 α on proline residue (Pro-OH). The von Hippel-Lindau (VHL) protein binds to HIF-1 and subsequently recruits a ubiquitin E3 ligase. 26s proteasome enzyme then degades HIF-1 α protein as a result of polyubiquitination. In the presence of oxygen, factor inhibiting HIF-1 (FIH-1) hydroxylates HIF-1α on an asparagine residue (Asn–OH). This tep inhibits binding of HIF-1α by the coactivator protein p300 resulting in prevention of HIF-1α from activating gene transcription (Figure 2.2 A).

Under hypoxic conditions (Figure 2.2 B), the Pro and Asn hydroxylation reactions are inhibited. The HIF-α (i.e., either HIF-1α or HIF-2α) rapidly accumulates, dimerizes with HIF-1β and recruits p300, which binds to hypoxia response elements, and activates the transcription by RNA polymerase II (Pol II) of hundreds of target genes, such as EPO encoding genes; VEGF and pyruvate dehydrogenase kinase 1 (PDK1) encoding gene, encoding pyruvate dehydrogenase kinase 1, which inhibits the conversion of pyruvate to acetyl coenzyme A for oxidation in the mitochondrion [1].

2.3 EPO receptor & downstream signalling

EPO receptor (EPO-R) is a 66-kDa polypeptide membrane protein that is a member of the cytokine receptor superfamily [25]. EPO-R is present on the surface of erythroid progenitors as a homodimer, even in the absence of ligand. On binding to EPO, the receptor undergoes a conformational change that brings its intracellular domains into close apposition enabling cross phosphorylation via the binding of Janus Kinase 2 (JAK 2) and the initiation of the signal transduction cascade (Figure 2.3).

Proteomic analyses of biotin- EPO/EPO-R complexes have identified transferrin receptor 2 (TFR 2) as an EPO-R partner. In UT7epo cells, TFR 2 facilitates EPO-R processing and transport to the cell surface. Erythroid progenitor cells from *TFR 2* knockout mice exhibit decreased EPO-sensitivity and CFU-E formation. During iron deficiency, TFR 2 also acts to balance erythrocyte production with available iron. Beyond its established roles in hepatocyte iron transport, TFR 2 also, therefore, modulates EPO-dependent erythropoiesis.

Transferrin receptor 1 (TFR1) can also modulate EPO-R signalling. Specifically, TFR1 ligation by polymeric-Immunoglobulin A 1 (p-IgA1) in murine erythroblasts increases EPO/EPO-R dependent mitogen-activated protein kinases (MAPK) and phosphoinositide 3- kinase (PI3K) signalling. This occurs in the absence of transferrin binding but depends upon a TFR1 endocytic motif [26].

Protein regulator of human erythroid cell expansion (RHEX) has recently been described as a new EPO-R associated factor. It promotes erythroid progenitor expansion and late-stage haemoglobinised erythroblast development. In UT7 / epo cells, RHEX is associated with EPO-R/JAK2 complexes, and its tyrosine phosphorylation is strongly induced up to >20 fold by EPO exposure. In primary erythroid progenitor cells, RHEX exhibits lineage-restricted expression, and its knockdown attenuates extracellular signal-regulated kinases (ERK) 1/2 activation as well as late-stage human erythroblast development [27].

Figure 2.3. EPO- Receptor signalling and modulating factors [26]

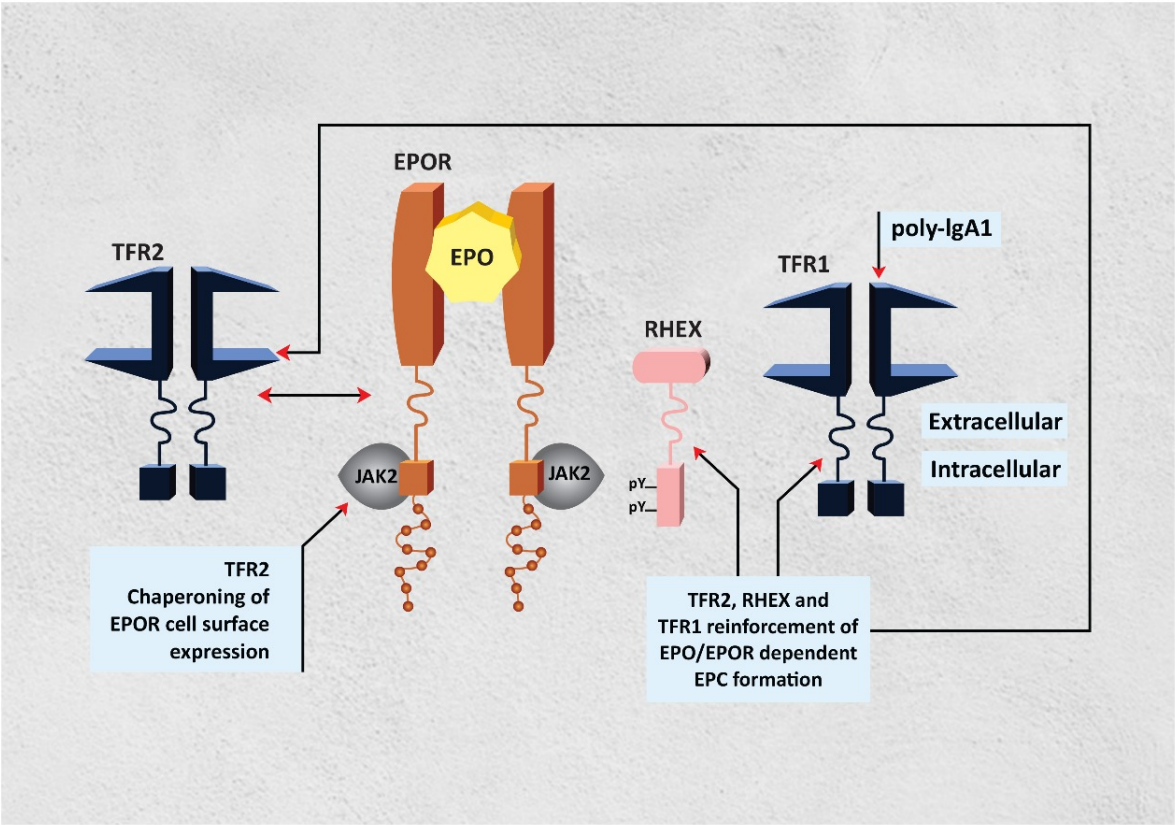


Figure 2.3. On binding to EPO, the EPO receptor undergoes a conformational change that enables cross phosphorylation via the binding of Jak2 kinase and the initiation of the signal transduction cascade. TFR 1 and TFR 2 also modulate EPO receptor signaling. RHEX is associated with EPO R and promotes EPO dependent erythroblast formation.

JAK 2 phosphorylation creates the docking site for signalling adaptors & mediators. Upon activation of EPO-R, multiple signalling molecules / pathways are activated. The biological consequences attributed to these pathways include anti-apoptosis, proliferation and differentiation. Prominent pathways include PI3 Kinase /protein kinase B (Akt), *RAS/RAF*/Mitogen-activated protein kinase (MAPK) and signal transducer and activator proteins (STAT) 5 pathway.

EPO/EPO-R/STAT 5 activation leads to transcription of B-cell lymphoma extra large (Bcl-xL) gene [28]. This pathway is seen as a prominent downstream pathway of EPO-R and its antiapoptotic properties probably explain major viability response associated with EPO action[29]. However, in in-vitro studies, EPO can efficiently protect Bcl-xL knockout erythroid progenitor cells. Hence raising a possibility of additional mediators in EPO-EPO R associated cytoprotection [30].

Intracellular Spi 2A serpin (Serine protease inhibitor) has been identified as new EPO/EPO-R/STAT 5 target and cytoprotective factor. It inhibits B- & L-cathepsins which when leached from damaged lysosomes can trigger apoptosis. EPO/EPO-R/STAT 5 also induce expression of a cytokine called erythroferrone (ERFE) which leads to reduced hepcidin production subsequently leading to better iron availability for erythropoiesis [26].

EPO/EPO-R/RAS/RAF /MAPK pathway

Binding of Src homology 2 domain containing transforming protein 1 (SHC) / growth factor receptor-bound protein (GRB) 2/son of sevenless homolog (SOS) complex to activated EPO-R eventually leads active MAPK to the nucleus. This results in phosphorylation of transcription factors such as ETS domain-containing protein gene (ELK-1) that promote cell cycle progression and proliferation. The Phosphoinositide phospholipase pathway is also activated via EPO-R and appears to feed predominantly into RAS/RAF/MAPK pathway promoting proliferation.

EPO/EPO-R/PI-3 kinase/Akt pathway

The Phosphoinositide-3 kinase (PI-3 kinase) is recruited to EPO-R by binding of the p85 regulatory subunit to the EPO-R. PI-3 kinase /Akt pathway has been implicated in controlling both proliferation and differentiation responses of erythroid cells. Activated PI3-kinase and Akt activated by EPO, phosphorylates

and activates GATA-1.

Active GATA -1 promotes the establishment of an erythroid phenotype and suppresses alternative lineage / multipotent progenitor capacity of the cells through the positive feedback loops where GATA-1 promotes EPO-R expression. Further degradation and loss of GATA-1 appear to be required at the late stages of erythroid differentiation, illustrating stage-specific effects of many intrinsic and extrinsic factors involved in erythropoiesis.

Growth factors: other cytokines
Cytokines such as stem cell factor, interleukin 3, GM CSF and insulin-like growth factor 1 enhance erythropoiesis in the presence of EPO.

3 Iron Metabolism

Iron is a vital element required for energy production, oxygen utilisation and cellular proliferation. Although all cells require iron, quantitatively most of the iron in the body is found in erythroid cells and most of the daily movements of iron cycles through the erythroid compartment.

Iron is a tightly regulated metal as deficiency and excess can lead to anaemia and haemochromatosis respectively. There is no iron loss from the body except in the presence of blood loss. Most of the iron transported to erythroid cells is directed towards the mitochondria for heme synthesis. Heme (ferrous protoporphyrin IX) is a planar molecule consisting of an atom of ferrous iron in the centre of a tetrapyrrole ring. Most of the heme is then bound to alfa or beta globin subunits that combine to form alfa-beta dimers that in turn join to form the functional alfa-2 beta-2 tetramer of haemoglobin.

Iron is absorbed primarily from duodenum where enterocytes absorb iron from the diet through the divalent metal transporter (DMT) -1 receptor. Enterocytes then export iron to circulation in ferric form. Transferrin transports iron in a soluble form to erythroid bone marrow or other iron requiring cells expressing

TFR 1 receptor. Cells regulate the absorption of transferrin-bound iron by altering TFR 1 on the cell surface. Reticuloendothelial macrophages are involved in iron storage and recycling through the breakdown of senescent RBCs. Reticuloendothelial macrophages provide a significant source of iron (25-30 mg).

Hepatocytes play a central role in iron regulation through secretion of Hepcidin to iron concentration in the body as well as storage and release of surplus iron. Iron export from all the cells occurs through the basolateral transporter called ferroportin.

Ferroportin is post-translationally regulated by hepcidin. Hepcidin acts as central iron-regulating hormone which leads to endocytosis of Ferroportin leading to reduced availability of iron in the circulation. The consequent iron retention in enterocytes reduces iron absorption, and a similar effect happens in reticuloendothelial macrophages leading to decreased iron turn over. Hepcidin is upregulated in response to increased transferrin saturation, inflammation, infection and endotoxins. Hepcidin is downregulated in iron deficiency, ineffective erythropoiesis, hypoxia, increased levels of erythropoietin or growth differentiating factor (GDF) 15 [11]. Hepcidin regulation in hepatocytes is thought to occur through the following major pathways:

1. Iron status: Bone morphogenic protein 6 (BMP-6) produced by hepatocytes and enterocytes in response to iron availability, acts by binding to BMP type 1 & 2 receptor while interacting with co-receptor hemojuvelin (HJV). This leads to phosphorylation of SMAD 1/5/8 which then interacts with SMAD 4 resulting in a transcriptional complex that activates hepcidin production in the nucleus. Circulating iron signal is provided by transferrin binding to TFR 1 & 2. Hepcidin production is modulated by the binding of these receptors to haemochromatosis protein (HFE).

2. Inflammation: Inflammatory cytokine such as IL-6 induce hepcidin expression through JAK signal transducer to phosphorylate STAT-3, which in turn interacts

with the promoter of the hepcidin gene in the nucleus[31].

3. Hypoxia: Under hypoxic conditions, HIF transcription factors upregulate expression of TMPRSS6 (transmembrane protease, serine 6; matriptase-2) which inhibits the action of BMP 6 by cleaving HJV from the cell membrane.

4. Erythropoietin: Erythropoiesis drive stimulated by erythropoietin down regulates hepcidin in a dose-dependent manner [32]. TFR 2 in association with EPO-R is involved in the production of GDF 15, a suppressor of hepcidin production by hepatocytes which in turn leads to greater iron availability[33].

Figure 3.1 Hepcidin regulation

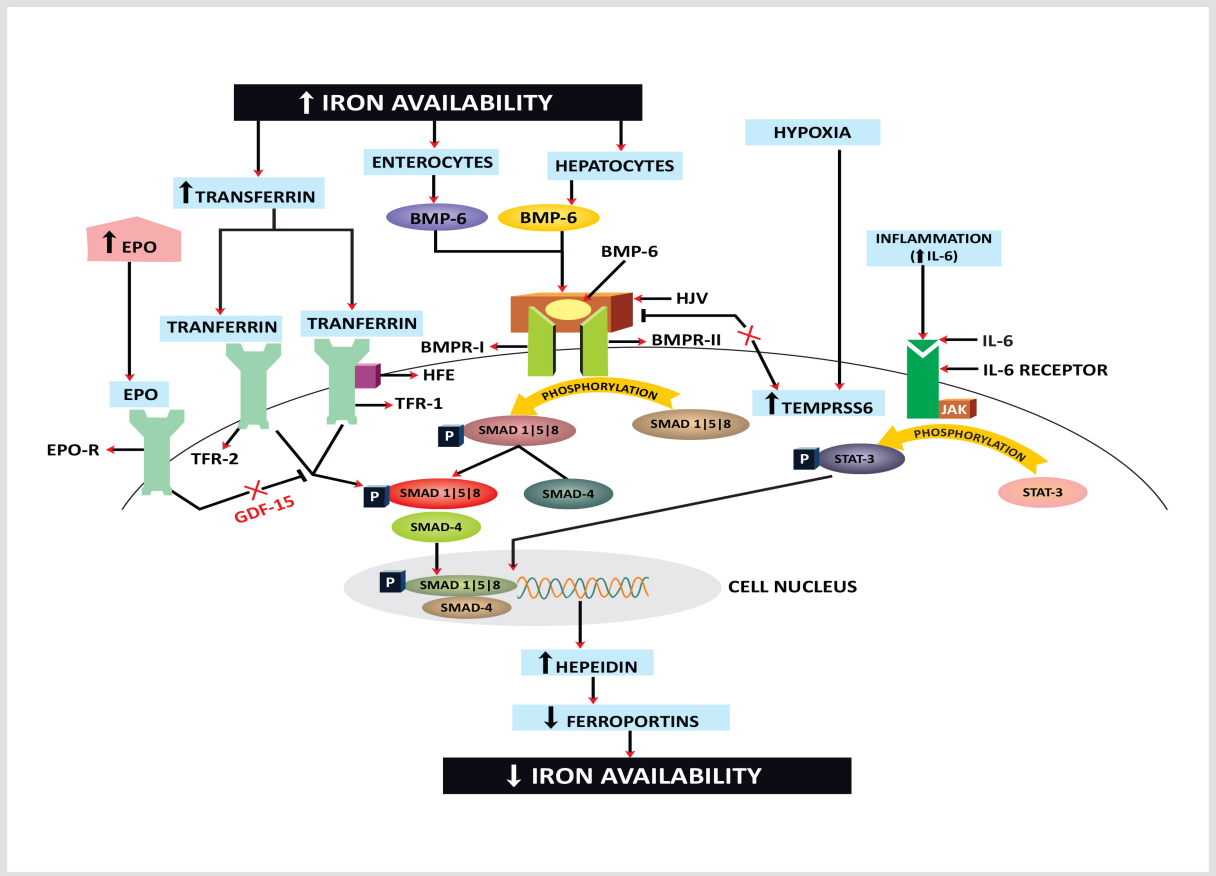


Figure 3.1: Hepcidin regulation

Positive regulation: Increased iron availability leads to upregulation of Hepcidin synthesis through BMP 6 pathway and transferrin receptors. Inflammation (IL-6) also induces hepcidin expression through JAK signal transducer leading of phosphorylation of STAT-3.

Negative regulation: Increased EPO leads to Hepcidin suppression by GDF 15 production through TFR 2 which is associated with EPO-R. Hypoxia leads HIF transcription factor to increase production of TMPRSS6 which in turn inhibits BMP 6 mechanism by cleaving HJV associated with Bone morphogenic receptor (BMPR) 1 & 2.

4 Anaemia and chronic kidney disease

Richard Bright first observed Anaemia in chronic kidney disease patients in 1836 at Guy's Hospital who stated that 'after a time, the healthy colour of the countenance fades'[34].

As chronic kidney disease progresses, there is an increasing prevalence of anaemia. Anaemia in CKD is typically normochromic, normocytic and hypo proliferative. In 1948 and early 1950s erythropoietin was identified as a humoral factor which stimulated red cell production. Subsequently, in 1955 the first quantitative and specific assay for EPO was developed [35]. In 1957, kidneys were identified as the primary site of EPO production in animal studies [36]. Subsequently in the late 1970s to mid-1980s, purification and cloning of EPO led to the development of immunological assays for quantifying the level of circulating EPO [37, 38]. EPO levels were considered inappropriately low in patients with anaemia with CKD compared to anaemic patients with normal kidney function who had 10-100 times higher EPO levels [39].

Availability of EPO and iron in bone marrow are essential for red cell production. Anaemia in CKD is primarily because of reduction of EPO production by peri-tubular fibroblast cells in the kidney. Available iron at bone marrow is also one the major factors which affect red cell synthesis in CKD patients. As described above systemic and cellular iron levels are very tightly controlled. CKD patients on haemodialysis have increased iron loss of approximately 1-3 gm per year due to chronic bleeding secondary to uraemia associated platelet dysfunction, blood loss in haemodialysis circuits and frequent blood tests [39]. Therefore, iron therapy remains a mainstay of treatment for anaemia of CKD along with EPO therapy.

5 CKD & Inflammation

Inflammation is prevalent among Haemodialysis patients [40]. Chronic kidney disease is known to be a state of sterile inflammation. This generalised increase in inflammatory response in particular with haemodialysis patients occurs because of various factors including decreased clearance of pro-inflammatory cytokines, endotoxemia as a result of volume overload, oxidative stress, old unused arteriovenous fistula or grafts, biofilm in dialysis catheters, old kidney transplants and blood dialyser interface.

Elevated cytokine levels in CKD patients could be as a result of decreased clearance or increased production. A study of 176 patients with advanced CKD (median GFR, 6.5 ± 0.1 mL/min) analysed the relationship between inflammation and severity of renal function impairment. Level of inflammation was measured by highly sensitive C-reactive protein (hs-CRP), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), hyaluronan, and neopterin levels checked after overnight fasting. There were significantly higher levels of hs-CRP, hyaluronan, and neopterin levels in the subgroup with lower GFRs. Statistically significant negative correlations were also noted between GFR and IL-6 (rho = −0.18; $P < 0.05$), hyaluronan (rho = −0.25; $P < 0.001$), and neopterin (rho = −0.32; $P < 0.0005$) [41, 42]. This study favours the theory of reduced clearance of cytokines in advanced CKD patients.

Chronic fluid overload also contributes to the burden of inflammatory status in CKD patients. In haemodialysis patients with loss of residual urine output, chronic fluid overload is commonly seen in practice. Studies in patients with fluid overload due to congestive cardiac failure have shown increased levels of pro-inflammatory cytokines and bacterial endotoxins [41, 43].

In CKD patients gut flora is altered due to dietary restrictions, and there is a change in enteric permeability as a result of fluid overload. Hence gastrointestinal tract is another source of chronic inflammation in CKD. Gut bacterial deoxyribonucleic acid (DNA) fragments have been detected in the

blood of both pre-dialysis CKD and chronic haemodialysis patients. Using D lactate as a marker of gut permeability, Shi et al. detected bacteria which were mainly of gut origin in plasma of 12 out of 52 chronic dialysis patients using 16s ribosomal DNA amplification and pyrosequencing. The presence of bacteria correlated well with CRP and IL-6 levels [44].

Dialysis is associated with increased generation of oxidants which in turn gives rise in cytokine production. The rise in the markers of oxidative stress correlated well with CRP levels in haemodialysis patients in a study by Levin et al. [45].

Haemodialysis access is an important contributor to the inflammatory milieu of haemodialysis patients. Central vascular catheters (CVC) and arteriovenous grafts compared to arteriovenous fistula (AVF) are associated with the greater burden of inflammation and mortality among haemodialysis patients [46, 47]. A small study also demonstrated the presence of infection in old clotted unused grafts even in the absence of clinical signs of infection among haemodialysis patients [48]. Failed transplant grafts in patients on haemodialysis are also shown to be associated with inflammation and EPO resistance. Transplant nephrectomy is associated with amelioration of markers of chronic inflammation [49].

Currently, there is no consensus regarding the measurement or assessment of the degree of inflammation in CKD patients. Most of the guidelines recommend individual patient approach towards monitoring inflammation among haemodialysis patients. Acute phase reactants such as highly sensitive CRP and proinflammatory cytokines such as IL -6 levels correlate with other markers of inflammation in CKD patients[50].

6. Consequences of inflammation in CKD

6.1 ESA resistance

Unfortunately, about 10-20% of haemodialysis patients show resistance or inadequate response to ESAs and hence require higher dosage. High ESA requirement is associated with increased cardiovascular mortality and morbidity [51, 52]. Moreover, the health economic consideration for the treatment of ESA-resistant patients with ever-increasing amounts of ESA was also very significant. In 2010 as a part of the internal audit in Barts Health NHS Trust, the cost for ESA alone for 250 ESA-resistant patients amounted to £2.5 million per year which is the same cost as for the remaining 950 ESA-sensitive patients. Fortunately since the adoption of tendering process in our unit, the purchase price of ESA has fallen but still cost of care of such group of patient is high due to their frequent hospitalisations.

Chronic inflammation is one of the major factors associated with ESA hyporesponsiveness in patients with CKD and end stage renal disease (ESRD)[53-55]. Higher ESA requirements are indicative of the risks associated with the potential cause of ESA resistance such as high inflammation burden and non-erythropoietic effects of higher ESA dose.

Several studies have also shown that there is a correlation between elevated levels of cytokines such as TNF-α, IFN-γ, IL-1, IL-6 and erythropoietin resistance in haemodialysis patients [56-58]. Pro-inflammatory cytokines inhibit erythroid progenitor cell proliferation and antagonise the anti-apoptotic action of erythropoietin [56, 59]. The direct negative effect on erythroid progenitors is primarily due to alterations in the sensitivity to EPO. TNF-α and IL-1 affect EPO synthesis *in vitro* and cause a dose-dependent inhibition of hypoxia-induced EPO production in the Hep3b cell line [60].

Hepatic hepcidin synthesis increases due to inflammation as a consequence of IL-6 induction, mediated through BMP signalling. Raised hepcidin concentrations

lead to functional iron deficiency as a consequence of down regulation of ferroportin resulting in intracellular accumulation of iron in macrophages and enterocytes. This functional non-availability of iron for erythropoiesis leads to impaired haemoglobin synthesis [61].

Earlier work in our laboratory by Allen *et al* showed that in an in-vitro study, CFU-E colony growth was suppressed when incubated with serum from inflamed uraemic patients compared to those incubated with serum from control subjects. The effect was reversed in a similar co-culture experiment by adding neutralising polyclonal antibodies against TNF-α and IFN-γ. This experiment suggests a direct correlation between pro-inflammatory cytokines and ESA response [62].

High ESA requirement is associated with poor outcomes and high cardiovascular morbidity and mortality in patients on haemodialysis [63]. Therefore addressing ESA resistance is a promising treatment option for patients with ESRD.

Various adjuvant therapies have been investigated in the past such as L-carnitine, vitamin C and E in order to treat ESA hyporesponsiveness but unfortunately, there is no clear evidence to support any of these interventions. This finding has been confirmed by a recent Cochrane review that concluded that there is inadequate evidence to recommend any intervention for ESA resistance. Further, adequately powered randomised controlled trials (RCT) are therefore required [64].

6.2 Cardiovascular risk

Over the last two decades, inflammation has been implicated as a risk factor for developing atherosclerosis and subsequently leading to plaque rupture and developing the dreaded complications such as acute coronary syndrome and stroke [65, 66]. In animal studies, leukocyte recruitment and expression of pro-inflammatory cytokines characterise early atherogenesis, and the inhibition of inflammatory mediators mutes atheroma formation[67]. Inflammation in CKD patients undergoing haemodialysis is associated with poor outcomes due to enhanced cardiovascular risks and mortality[68, 69].

There is a very high prevalence of coronary artery disease in CKD patients. The severity of obstructive coronary artery lesion rises with increasing severity of disease [70-72]. Traditional risk factors are present in most of the incident advanced CKD patients. These risk factors do not explain the higher incidence of cardiovascular disease in CKD patients as previously known tools which use traditional risk factors for predicting cardiovascular risks such as Framingham equation demonstrate poor overall accuracy in predicting cardiac events in individuals with CKD[73].

In a prospective study of 80 non-diabetic uraemic pre-dialysis patients, the relationship between markers of inflammation & oxidative stress such as plasma levels of CRP, fibrinogen and advanced oxidation protein products and the incident first occlusive cardiovascular event was studied. At a median follow up of seven years, adverse outcomes in all 21 patients were independently associated with age and markers of inflammation and oxidative stress [74]. Similarly, in a study of 45 haemodialysis patients observed over 12 months showed worsening of carotid intima-media area in common carotid artery was associated with elevated IL-6 levels[75].

A study of a historical cohort of 393,451 US dialysis patients demonstrated that septicaemia resulting in inflammation was associated with increased cardiovascular deaths[76]. There is evidence to support that inflammatory state measured by elevated acute phase reactants is associated with increased cardiovascular morbidity & mortality among CKD patients [77, 78]. Therefore inflammation may be the missing link in cardiovascular risk prediction in patients with advanced CKD.

7. Cardiovascular morbidity and mortality in CKD patients

Apart from CKD being an independent predictor of high cardiovascular risk, patients with CKD usually have a very high burden of comorbidities such as diabetes, hypertension and chronic inflammation predisposing them for atherosclerotic disease process[79].

Cardiovascular events occur as a result of atheromatous plaque rupture in the arterial wall, which leads to narrowing or occlusion of the vessel lumen. This leads to downstream catastrophic ischaemia resulting in myocardial infarctions, strokes and critical limb ischaemia. However, atherosclerotic disease leading to vaso-occlusive events such as acute myocardial infarction itself does not account for the majority of cardiovascular mortality in patients with advanced CKD and ESRD. This is also demonstrated by the poor outcomes following PCI in ESRD patients compared to non-CKD patient cohort [79].

Congestive cardiac failure, arrhythmias and sudden death account for the major cause of mortality and morbidity in patients with CKD and ESRD patients as published in recent United States Renal Data System (USRDS report in 2016. 74% of patients with CKD 5 have left ventricular hypertrophy (LVH) at the time of initiation of haemodialysis. Pathogenic factors for LVH in patients with advanced CKD and dialysis include chronic volume overload, intradialytic fluctuation of circulating volume, anaemia and arterioscelrosis [80]. Both atherosclerosis and arteriosclerosis contribute to cardiovascular morbidity in CKD patient through overlapping mechanisms.

7.1 Atherosclerosis

Development of atheromatous plaques is referred to as atherogenesis, which in turn leads of atherosclerosis. Atherogenesis starts as changes in endothelial layer of blood vessels. The endothelial cells when subjected to irritative stimuli such as dyslipidaemias, hypertension or pro inflammatory cytokines start

expressing adhesion molecules such vascular cell adhesion molecule 1 (VCAM-1) for leucocytes. Once adherent to endothelium chemo attractant molecules direct transfer of the leucocytes (majority monocytes), which penetrate into the innermost layer of artery tunica intima. Growth factors such as macrophage colony stimulating factor 1 stimulate blood derived monocytes in the arterial wall differentiate into tissue macrophages [81].

Changes in endothelial permeability and the composition of extracellular matrix beneath the endothelium promote the entry and retention of cholesterol containing low-density lipoprotein particles (LDL) in the vessel wall particularly in the areas of strain. Phospholipids produced as a result of modification of LDL can induce endothelial cells to express leucocyte adhesion molecules.

The macrophages in the intima have scavenger receptors for LDL particles. Macrophages engulf lipoprotein particles and resulting in the formation of foam cells. The macrophages in the atheroma may also have a pro inflammatory function by release of inflammatory cytokines and free oxygen radicals. T cell activation as a result of various antigens presented with in the atherosclerotic plaque leads to production of interferon gamma (IFN-γ), which activates macrophages and vascular cells leading to further inflammation in the plaque [82]. Macrophage accumulation largely define the fate of an atherosclerotic plaque and is proportional to plaque size [83]. Inflammation leads to Akt/protein kinase B activation of hexokinase-1 and hexokinase-2 in mitochondria and inhibit pro apoptotic Bcl-2 associated X protein (Bax). This pathway prevents apoptosis consequently increasing macrophage population in the plaque.

Smooth muscle cells (SMCs), which are presented in tunica media, are recruited into intima as a result of inflammatory stimulus with in the atheromatous plaque. In the intima SMCs produce extracellular matrix molecules such as interstitial collagen and elastin and form a fibrous cap of that covers the plaque. The cap overlies a collection of foam cells, accumulation of cellular debris and extracellular lipids, which form necrotic core of the plaque.

Increase in atherosclerotic plaque size causes complications by producing narrowing of the vessel lumen resulting in downstream ischaemia or catastrophic occlusion of the vessel lumen as result of plaque rupture causing thrombosis. The activated immune cells in the atherosclerotic produce inflammatory cytokines resulting in production of IL-6. IL -6 in turn stimulates production of acute phase reactants such as CRP and serum amyloid A [66, 84-86].

7.1.1 Quantification of atherosclerotic plaque inflammation

Currently in clinical practice atherosclerosis can only be assessed by angiography of the blood vessels, which measures intraluminal stenosis of the blood vessel. However, this approach does not provide any indication of plaque activity or overall inflammation load in the vasculature. Newer disease-modifying treatments such as statins act upon plaque inflammatory activity leading to plaque stabilisation.

18 Fluorodeoxyglucose (FDG) positron emission tomography (PET) [87, 88] combined with computed tomography scan (CT) is a molecular non-invasive imaging technique which is highly sensitive to locate metabolically active processes [89]. After intravenous injection, 18 FDG is taken up by glucose transporters (GLUT 1 &3) into the cells [90]. In the cytosol, 18 FDG is phosphorylated by hexokinase into 18 FDG-6-phosphate, which cannot undergo glycolysis because it lacks the necessary 2'hydroxyl group and remains trapped within the target cells where it accumulates in proportion to metabolic demand.

In a landmark study, Rudd *et al*. demonstrated higher metabolic activity in atherosclerotic plaque in symptomatic carotid artery disease measured with 18 FDG intake compared to asymptomatic plaque [91]. Arterial 18 FDG PET CT scan allows quantification of arterial inflammation and has a significant role in monitoring the response of atherosclerosis inflammation to intervention [87] [92].

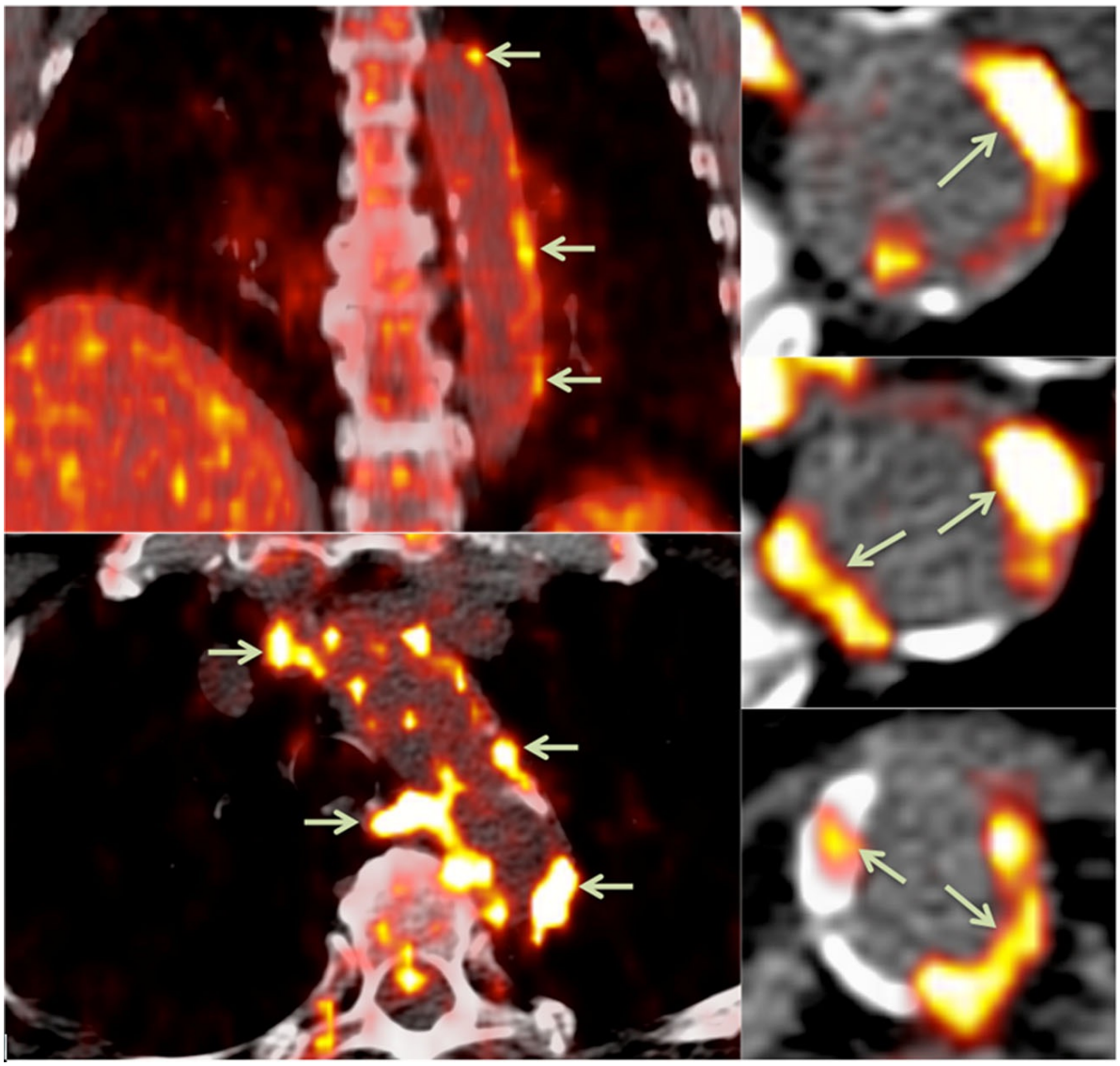
The vascular FDG uptake occurs as a result of increased pro-inflammatory macrophage metabolic activity leading to increased glucose turnover facilitated by up regulation of glucose transporter protein (GLUT) transporters within the macrophages. Ex vivo studies have shown the histological correlation between 18 FDG uptake and macrophage density as well as increased gene expression markers of glycolysis and inflammation.

FDG signal is likely to be most prominent in the early stages of atherosclerosis, during foam cell formation and subside after plaque calcification is established. Arterial 18 FDG PET does not usually co-localise with calcification seen on the CT portion of combined PET-CT scan. Hence supporting the concept that inflammation and calcification occur at different stages in atherosclerosis.

The degree of 18 FDG uptake correlates with many of cardiovascular risk factors as well as with Framingham cardiovascular risk scores, inflammatory biomarkers (such as CRP, Matrix metalloproteinases (MMPs) and adeponectin), gene expression markers of glycolysis and inflammation. Aortic inflammation detected by 18 FDG PET is also related to increased aortic stiffness determined by aortic pulse wave velocity, which is a reliable prognostic indicator of cardiovascular events.

PET imaging can provide a global measure of vascular inflammation by averaging 18 FDG SUV readings over multiple segments of an index vessel (such as the aorta). Focused evaluation of a plaque could also be obtained from a single investigation (Figure 7.1) [93].

Figure 7.1: FDG PET CT image demonstrating focal atherosclerotic plaque FDG uptake along the wall of descending aorta (arrows)[94].



In a retrospective observational study of 513 cancer-free patients undergoing PET CT scan as a part of the oncological evaluation, aortic 18 FDG TBR strongly predicted cardiovascular events independent of traditional risk factors (HR 4.71, P<0.001) with 20 to 30 % net reclassification improvement over Framingham risk score alone in the highest risk group. Arterial FDG uptake along the ascending aorta measured as target to background ratio (TBR) provided information regarding the potential of a subsequent cardiovascular event as shown in Figure 7.2 [95].

Figure 7.2: Association between aortic wall inflammation and timing of cardiovascular events.

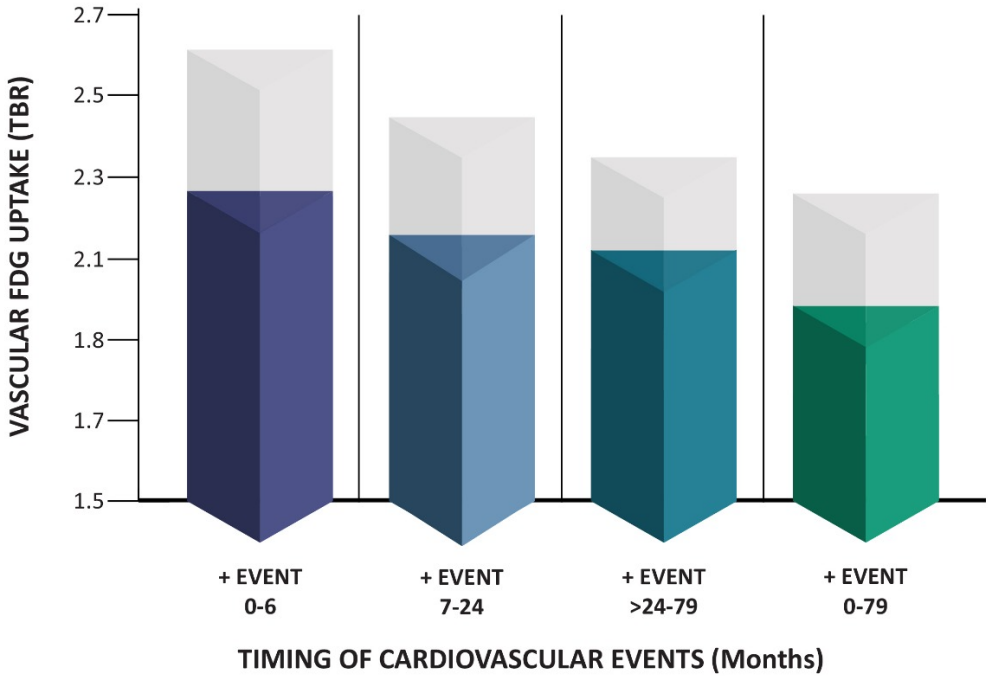


Figure 7.2. Association between aortic wall inflammation and timing of cardiovascular events [95] The bar graphs show mean (standard deviation) TBR in relation to timing of cardiovascular events in months and no events at 79 months from the time of scan.

Serial 18 FDG plaque imaging has been used to evaluate changes in atherosclerotic plaque inflammatory activity as a result of intervention leading to a detectable reduction in vascular 18 FDG uptake. 18 FDG PET vascular imaging has been used as a surrogate endpoint in several clinical trials involving anti-atherosclerotic drugs such as statins [96, 97].

As discussed above traditional risk factor assessment tool such as the Framingham risk score are not valid in patients with advanced CKD. In a retrospective study with 64 patient with Stage 3 chronic kidney disease and 64 controls undergoing FDG PET CT scan, CKD is associated with increased arterial wall inflammation[98]. Measurement of plaque inflammation with 18 FDG PET CT vascular imaging has a potential to become a useful prognostic marker for cardiovascular risk.

7.2 Arteriosclerosis

Arteriosclerosis or arterial wall stiffening of aorta resulting in elevated afterload is a major contributor to cause of LVH, diastolic dysfunction, impaired coronary artery flow during the diastolic phase in CKD patients[99].

Arterial stiffness is a condition, which is commonly noticed in patients with CKD and ESRD. Other risk factors associated with increased aortic stiffness are age, hypertension and diabetes [100-102]. A recently published large observational study with 2933 participants with CKD observed over four years failed to show a correlation of baseline level of inflammation with arterial stiffness during the study period. One of the criticisms of this study was that single baseline measurement of the study might not reflect true degree of inflammation as it fluctuates over a period [103].

Other studies investigating inflammatory conditions such as rheumatoid arthritis have established a correlation between the systemic inflammatory state with aortic stiffness and vessel wall inflammation. In small studies, interventions to reduce inflammation such as anti TNF-α therapy in patients

with rheumatoid arthritis have resulted in improvement of arterial stiffness [104] [105].

Arterial stiffness or arteriosclerosis is associated with end-organ damage and subsequent cardiovascular adverse events as a result of various risk factor exposure. Arterial stiffness is associated with wide pulse pressure which gets transmitted to microvasculature resulting in increased pulse wave velocity leading to reflective wave causing increased after load & LVH. This resulting tensile stress can lead to microvascular damage, endothelial dysfunction and resulting chronic inflammation[106]. Aortic stiffness leads to loss of delicate balance between of physiologically matched aortic distensibility which accommodates for stroke volume blood flow from the left ventricle into aorta during systole and smooth forward blood flow during diastole. Increase in cardiac afterload as a result of aortic stiffness leads to LVH and dilatation[107].

Aortic stiffness is characterised by thickening and concentric calcification of the medial arterial layer in CKD. Hyperplasia and hypertrophy of smooth muscle cells and increased collagen tissue in the arterial wall also contribute to loss of arterial elasticity. The exact mechanism of increased arterial stiffness in CKD is still uncertain. However, the role of advanced glycated products, endothelial dysfunction, renin-angiotensin aldosterone system (RAAS), vascular calcification, disorders of bone and mineral metabolism and chronic inflammation have been shown to be associated with increased arterial stiffness in CKD patients [108].

In one of the first studies establishing aortic stiffness as a predictor of all cause and cardiovascular mortality, London et al. conducted a study on 241 subjects undergoing haemodialysis over a period of 10 years. Aortic pulse wave analysis was measured by ultrasonography along with echocardiogram and other lab parameters, at the time of entry in the study. Age and aortic pulse wave velocity (PWV) were noted to be predictors of all cause and cardiovascular mortality based on Cox analysis. For cardiovascular mortality, Odds ratio for PWV >12.0

versus <9.4 m/s was 5.9 (95% CI, 2.3 to 15.5). For all-cause mortality, the odds ratio was 5.4 (95% CI, 2.4 to 11.9). In the given population, for each increase of 1m/s in the PWV, the all-cause mortality adjusted OR was 1.39 (95% CI, 1.19 to 1.62) [109].

7.2.1 Aortic stiffness quantification

Carotid-femoral pulse wave velocity measurement by applanation tonometry was studied and proven to be a surrogate marker for adverse cardiovascular outcomes despite interobserver variability [109, 110].

The Dallas heart study has shown that measurement of aortic stiffness by cardiac MRI allows a measure of total arterial compliance (TAC), a measure of global arterial stiffness and ascending aortic distensibility (AD) and aortic arch pulse wave velocity (PWV), a measure of aortic stiffness. Total arterial compliance and aortic distensibility may be stronger predictors of cardiac adverse events. Pulse wave velocity is considered to be a predictor of nonfatal extra-cardiac vascular events. It was one of the pioneering studies to study the association of aortic stiffness measured with CMR with adverse cardiovascular outcomes[111].

CMR allows good repeatability of the scans with precisely placed imaging planes. Interobserver variability is also minimised as a result. Full visualisation of the vessel allows studying different sections of the aorta, which may have different prognostic value. When the heart is also studied in the CMR protocol, the accurate assessment of myocardial function and could be made which is directly associated with aortic stiffness. However, MRI scan is limited by its contraindications such as presence of pacemakers and claustrophobia. It is an expensive test which is not widely available.

7. Pentoxifylline

There is emerging evidence for Pentoxifylline, a non-selective phosphodiesterase inhibitor as a potential treatment for ESA hypo-

responsiveness. It is currently licenced for the treatment of peripheral vascular disease. Pentoxifylline is associated with a reduction of pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-6 in haemodialysis and non-dialysis dependent patients with CKD as well as improving haemoglobin status [112, 113]. Before PEAR study was planned, the evidence in favour of Pentoxifylline treatment was based upon following uncontrolled studies

- Navarro *et al*. treated 7 anaemic CKD patients not on dialysis with Oxypentoxifylline (oral 400 milligram (mg) daily) for 6 months. Haemoglobin levels significantly increased from 9.9 ± 0.5 to 10.6 ± 0.6 g /decilitre (dl) ($p < 0.01$).[113]

- A study by Cooper *et al.* showed that Pentoxifylline administration resulted in improvement of haemoglobin in ESA resistant haemodialysis patients. 16 patients on chronic haemodialysis were given pentoxifylline 400 mg OD for 4 months. All patients had Hb of <10.7g /dl for 6 months before recruitment to the study while on ESA dose of >12000 International Units (IU)/week (wk). Mean Hb concentration increased from 9.5 ± 0.9 to 11.7 ± 1.0 g/L ($p = 0.0001$) for the 12 patients who completed the study. There was a statistically significant reduction in ex vivo T cell expression of TNF alfa and interferon gamma after treatment compared to baseline levels [8].

- In a study by Ferrari *et al*. published in 2010, 14 CKD patients (e GFR 23±6 ml/min) not on ESAs were treated with Pentoxifylline 400 mg daily for 4 weeks. Cause of CKD was non-inflammatory renal disease and none of the patients had received parenteral iron or immunosuppressive therapy. Total 10 patients completed the study. There was an increase in Hb from a baseline of 11.1±5 g/dl to 12.3±6 g/dl ($p <0.001$) by the end of study follow up period. This trial also demonstrated a reduction in IL-6 in the cohort from a baseline of 10.6±3.8 picogram (pg)/ml to 6.6 ± 1.6 pg/ml ($p<0.01$). There was also an improvement in transferrin saturations from baseline of 15±3% to 20±5% ($p<0.003$) and statistically non-significant reduction in ferritin levels compared to baseline.

This small study again supported the hypothesis of inflammation driven inhibition of erythropoiesis [112].

Subsequently in the first double-blind placebo controlled randomised controlled trial (RCT) by Gonzalez-Espinoza *et al.* (2012) showed that in 18 haemodialysis patients with matched controls, administration of Pentoxifylline 400 mg per day is associated with significant reduction ($p<0.05$) in concentration of TNF-α, IL-6 and C reactive protein (CRP) after 4 months of treatment. Haemoglobin or ESA dose changes were not an endpoint for this study. No significant adverse events were noted during the study [114].

The exact mechanism of action of Pentoxifylline in inflammation cascade is still not known. Phosphodiesterase inhibition leads to intracellular accumulation of cyclic adenosine-3,5-monophosphate (cAMP) resulting in activation of Protein kinase A (PKA). Activated PKA then leads to phosphorylation of the transcription factor cAMP-response element binding protein (CREB) and transmission of signals to the nucleus, and the subsequent modulation of gene transcription, contributing to down regulation of TNF-α production. Role of phosphodiesterase inhibitors in downregulating NF-kB transcriptional activity has also been explored[115, 116].

Effect of Pentoxifylline on ESA hypo-responsiveness could also be explained with the hypothesis that it could be exerting an inhibitory effect on hepcidin levels by the reduction in inflammation. The current clinical evidence is equivocal but there is a need for more focused studies to understand this aspect of Pentoxifylline [69, 117]. The available data suggest that Pentoxifylline in haemodialysis patients could be associated with the reduction in inflammation by reduction of pro-inflammatory and inflammatory cytokines leading to reduced ESA requirements.

8. Hypothesis

Anaemia is a common complication of Chronic Kidney Disease (CKD). Usually, the cause of anaemia in CKD is the reduced production of erythropoietin after other reasons such as iron deficiency, vitamins B12 and folate deficiency, inadequate dialysis, severe hyper-parathyroidism, myelosuppressive medications, active infection and haematological conditions are ruled out. Anaemia of CKD is treated with administration of erythropoietin stimulating agents (ESA). ESAs have revolutionised the treatment of renal anaemia since the late 1980's. Use of ESA is associated with better quality of life and a reduced need for blood transfusions.

Suboptimal hematologic response to treatment with ESA [51] remains a problem in considerable proportion of end stage renal disease (ESRD) patients on haemodialysis. Hyporesponsiveness to ESA has been attributed to chronic inflammation in CKD and ESRD patients. High ESA requirement and chronic inflammation are associated with increased cardiovascular morbidity and mortality in patients with ESRD.

Pentoxifylline administration has been shown to reduce inflammation in ESRD patients by down regulating the production of cytokines. Pentoxifylline could potentially reduce the burden of chronic inflammation in haemodialysis patients possibly resulting in reduced ESA requirement and improved cardiovascular outcomes in haemodialysis patients.

With this hypothesis, a single centre randomised placebo-controlled, double-blinded study was planned to assess the effect of Pentoxifylline on ESA dose, on surrogate markers of cardiovascular outcomes, safety and cytokine profile in clinically stable haemodialysis patients with high ESA requirement. Patients included in the study were clinically stable adult haemodialysis patients who had been requiring equivalent ESA dose greater than or equal to 6000 international units (I.U) equivalent of ESA per week or if ESA resistance index is greater than or equal to 6.5 I.U/kilograms(Kg) /Hb (gram/decilitre) per week. Haemoglobin (Hb) for equivalent ESA dose was between 9 to 12 gm / dl and patients were iron

replete before entering the study. Patients were randomised to receive either Pentoxifylline 400mg once a day or a matched placebo for six months in 1:1 ratio.

Randomisation was stratified for diabetes mellitus status and ESA requirement. The primary study end point was ESA requirement relative to haemoglobin level at 6 months. Secondary end points were safety analysis, Hb values, ESA doses and Cardiovascular imaging to assess the effect of pentoxifylline on changes in cardiovascular biomarkers after six months of intervention. Besides, cytokine profile was also analysed during the course of the study.

9. Methods & Materials

10.1 Aim

Aim of the study was to evaluate the impact of anti-inflammatory effects of Pentoxifylline on ESA responsiveness on ESA resistant haemodialysis patients. The trial was a double -blind placebo-controlled randomised study based at a single site. Patients were divided into four groups according to ESA requirements and diabetes status. In each group patients were randomly assigned to placebo or pentoxifylline in 1:1 ratio in blocks of 10 patients for each group. The patients who were randomised to experimental group received encapsulated Pentoxifylline sustained release 400 mg OD (Trental, manufactured by Sonafi Aventis imported from Portugal) while the patients randomised to placebo group received identical matching capsule for 6 months. Patients were supplied investigational medicinal product (IMP) every month.

Blood samples were taken during the study at the following time points
1) Weekly during run in period of 2 week for baseline value
2) Once monthly during the intervention period for 6 months
3) Fortnightly during wash out period of 1 month.
Total 11 visits were scheduled for the duration of study per patient.
FDG PET CT and Cardiac MRI scans were performed at baseline and 6 months.

Trial was conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations. Prior to initiation of the study ethical approval was obtained from the East London and City Research Ethic Committee and the study was approved by the Medicines Health Regulatory Authority (REC number: 12/LO/1635, EudraCT Number: 2011/006168/30). Trial was sponsored by Joint Research Management Office (JRMO) at Barts Health NHS Trust.

Another study was conducted in association of the PEAR study, named as PEAR study- experimental outcomes. As a part of this study, blood samples collected and stored for future research in PEAR study were later analysed for cytokine profile included Transforming growth factor (TGF) gamma, Tumour necrosis factor (TNF) alfa, Interferon (IFN) gamma, Interleukin (IL) 6, and IL-4. Ethical approval was obtained from the East London and City Research Ethic Committee.

10.2 Inclusion and Exclusion criteria

Inclusion Criteria

Eligibility for study participation was based upon data obtained from the most recent clinical visit(s). In addition, recent medical history was obtained prior to enrolment. The eligibility criteria for patient enrolment in the study was as follows:

- The subject should be able to read and understand the written consent form (with the help of a translator if necessary), complete study-related procedures, and communicate with the study staff.
- Willing to comply with study restrictions.
- Between 18 and 85 years of age (inclusive).
- Diagnosis of clinically stable ESRD, as determined by the investigator.
- Requiring regular dialysis therapy for at least 12 weeks prior to first administration of study agent.
- Last haemoglobin concentration at time of consent between 9.0 and 12.0 g/dL before entering the study.
- Receiving treatment with intravenous (I.V.) or sub-cutaneous (S.C.) erythropoietin stimulating agent (ESA) at least weekly (i.e. exclude Mircera™ or other ESAs given fortnightly or monthly) for a minimum of 8 weeks prior to administration of study agent, requiring doses to remedy ESA-resistance (requiring greater than or equal to 6000 International Units (I.U) equivalent of ESA per week or if ESA resistance index is greater than or equal to 6.5 I.U /kg/wk/g Hb for equivalent EPO dose), with evidence of stable Hb

concentrations.

- Serum folate and vitamin B12 levels concentrations which are checked annually as part of clinical practice, were normal and transferrin saturations greater or equal to 25% and / or ferritin > 200 µg/L (last result prior to consent), as per departmental protocol at The Royal London Hospital.

Exclusion Criteria

Ineligibility for study participation was based upon data obtained from the most recent clinical visit(s). In addition, recent medical history was obtained prior to enrolment. If a subject displayed any of the following criteria, he or she was not enrolled in the study:

- Clinically relevant abnormal history of physical and mental health other than conditions related to CKD of the patient, as determined by medical history taking (as judged by the investigator).
- Clinically relevant abnormal laboratory results, electrocardiogram (ECG), vital signs, or physical findings other than conditions related to CKD of patient (as judged by the investigator).
- Subject had uncontrolled hypertension (in the opinion of the clinician); subject was unable to refrain from the use of disallowed concomitant medication (such as immunosuppression / anti-inflammatory drugs) from one week prior to the first study drug administration until follow up assessments.
- Participation in an investigational drug trial in the 3 months prior to administration of the initial dose of study drug.
- Subject had undergone major surgery within 3 months prior to screening for eligibility for study participation.
- Females of child-bearing potential who were not willing to use contraception for the duration of the study.
- Females who were breast feeding.
- Subject known to be hypersensitive to the active constituent, Pentoxifylline, other methyl xanthines or any of the additives.
- Subjects with recent (3 months) cerebral haemorrhage, extensive retinal haemorrhage, acute myocardial infarction and severe cardiac arrhythmias.
- Contraindications to magnetic resonance imaging (e.g. severe claustrophobia,

pacemaker, defibrillators).

- Subjects who were on (or are due to start) immunosuppressive and anti-inflammatory drugs except asprin at a dose of ≤ 300 mg/d.
- Any other condition that in the opinion of the investigator would complicate or compromise the study (e.g. known haemoglobinopathy), or the wellbeing of the subject.

Premature withdrawal from the study

Systematic non-compliance to medications was the primary criterion for premature withdrawal from the study. End-point tests were performed (blood and radiology) if the patient agreed prior to formal withdrawal from the study. The 1 month safety follow-up period (blood tests) investigations were also performed if the patient agreed.

10.3 Intervention and study design

Placebo or pentoxifylline 400 mg taken once a day for 6 months.

Concomitant Therapy:

During the study, the patients continued all their regular therapy. Initiating a drug with known anti-inflammatory properties was avoided. If this had to be done because of the clinical condition of the patient, study drug administration was to be discontinued, and replacement of the patient was to be considered.

Concomitant medications were expected to include iron therapy on a regular basis to maintain T Sats >25% and/or ferritin >200 microgram (μg)/L, phosphate binders, statins, medication to treat hyperparathyroidism, anti-hypertensive agents and other drugs to treat known complications of renal failure. In general, concomitant medication resulting in non-eligibility included all medication that in the opinion of the investigator would complicate or compromise the study or interfere with the study objectives.

Study design:

This study was a single-centre, randomised double blinded placebo-controlled trial of 100 patients with ESRD on dialysis. Patients received either Pentoxifylline sustained release tablet 400 mg once a day or placebo for 6 months with a further 1month follow-up after therapy is stopped. Randomisation was stratified for diabetes mellitus status and ESA requirement (adjusted for Hb).

Randomisation process

1:1 randomisation was stratified for diabetes status and ESA requirement. Patients were classified into four groups based on ESA requirement and presence of diabetes. Patients were allocated into high ESA group if equivalent ESA requirement was >14,000 units / week. Patients were allocated to study drug according to recruitment sequence in each group. The drugs sequence had already been randomized by the investigational medicinal product IMP supplier. Patients in each group were randomised in blocks of 10 each. Patients and investigators were blinded to treatment allocation. Sealed unblinding envelopes were supplied with IMP.

Monitoring during the study

Subjects were entered in a "run-in" period for 2 weeks where blood tests were measured weekly to establish a baseline. All blood tests were taken prior to a dialysis session and preferably after an inter-dialytic gap of 48 hrs. If the ESA dose changed in the run-in period, the patients were withdrawn.

Following the run-in period, subjects were treated with either IMP or placebo for 6 months and they were reviewed monthly along with blood tests in addition to their routine monthly blood profile as per the local policy. IMP or placebo were provided to the patients during the monthly visits. Subjects were asked to return unused medication for compliance check.

After 6 months treatment (IMP or placebo) was stopped. Subjects were asked to return any remaining unused medication. Patients were reviewed fortnightly.

This "Off-Treatment Follow-up Period" lasted for one month. Changes in the ESA doses were done according to departmental guidelines.

Any adverse events were recorded and acted upon in accordance with pharmacovigilance guidelines of relevant supervising authorities.

10.4 Study Endpoints

Primary study endpoint was the ESA requirement relative to the Hb concentration.

Secondary endpoints included:

- Safety analysis.
- Hb values and ESA doses after 6 months of treatment.
- Cardiovascular imaging performed at baseline and at 6 months. The effect IMP had on the following cardiovascular parameters were examined:

a) Mean target-to-background ratio across a substantial portion of artery (typically aorta, supra aortic vessels and femoral arteries) using 18 Fluorodeoxyglucose (FDG) positron emission tomography 18-FDG-PET (time-of-flight). This allowed us to assess whether IMP has an anti-inflammatory effect on the blood vessels.

b) Vascular stiffness measures using magnetic resonance imaging

c) Measures of systolic and diastolic function, using cardiac magnetic resonance (CMR) tagging techniques (strain (%) and strain-rate (s-1).

Mechanistic end points:

Analysis of cytokine trends (IL-6, TNF alfa, TGF beta, IFN gamma) during the course of study.

10.5 Statistical considerations

Power of the study calculation:

From internal audit data on ESA usage, we assumed the average equivalent dose of ESA will be 12000 I.U./week (with standard deviation=500). If the placebo group have Hb=10g/dl, the ESA/Hb ratio = 1200. The study by Cooper *et al.* showed that 4 months treatment increased Hb levels by 25% [8]. We aimed to power this study to demonstrate that IMP will increase ESA response by 5%.

If we assumed the SD for ESA/Hb = 100, this would mean that for an alpha level of 5% (95% confidence interval) and a beta level of 5% (statistical power of 95%) we needed 30 patients in each group. We assume that there will be a 50% "drop out" (transplantation and patient withdrawal) so we planned to recruit 100 patients

Statistical analysis plan

Primary End-Point

The primary study endpoints was ESA requirement relative to Hb level. Statistical analysis was done by unpaired student t-test comparing values ESA dose per week (mcg) / Hb (in g/dl), Control vs IMP. P values of < 0.05 will be considered statistically significant
Conversion factor ESA IU = Aranesp dose in mcg x200

This was calculated for both:

1) Intention to Treat (ITT) Analysis
2) Per Protocol Analysis

For Intention to Treat, we will use last 2 available data points for patient that withdrew early from the study. For Per Protocol Analysis, we will use last 2 available data points when patient took their assigned treatment. If patients did not have more than 1 visit after randomisation, they will not be included in analysis.

Secondary end points

1. Safety analysis:

Overall adverse events (AE) and serious adverse events (SAE) rates were calculated based on the number of each event / duration subject was in the

study after randomisation. AE and SAE were categorised into categories as per case report form (CRF). The rates of AE/SAE for each category were calculated. Control vs Investigational medicinal product (IMP) rates were compared using Poisson regression, with no adjustment for multiple events per subject. Statistical significance if P-value <0.05 after Bonferroni correction was applied.

2) PET-CT

The following parameters were compared:
a) Standardised uptake value (SUV) max at end of study
b) Target to background ratio (TBR) at end of study
c) Δ SUVmax (percentage difference in SUVmax)
d) Δ TBR (percentage difference in TBR)

These parameters were compared on the ITT dataset using Student t-test.
P values of < 0.05 was considered statistically significant

3) CMR

The following parameters were compared:
a) Aortic compliance (area/systolic BP),
b) Left ventricular (LV) end diastolic volume
c) LV end systolic volume
d) LV systolic volume
e) LV ejection fraction
f) LV mass

These parameters were compared on the ITT dataset using Student t-test. P values of < 0.05 was considered statistically significant.

<u>Mechanistic end points:</u>

Cytokine levels were compared at baseline and follow up phase. Cytokine variability along the entire duration of study was also analysed as per protcol.

Baseline Demographics and Biochemical parameters

Continuous variables were tested for normality using *D'Agostino*-Pearson normality test. Mean (standard deviation) or median (interquartile ranges) were displayed. Statistical analysis was done by student t-test (unpaired) or Mann-Whitney U test. Categorical data was analysed by Chi Square. Statistical significance if P-value <0.05 after Bonferroni correction was applied

Demographic information:

a) Age at randomisation
b) Ethnicity: Categorical- White, Black, Indian sub-continent (Asians), Others.
c) Gender: Categorical- Male vs Female
d) Residual renal function at randomisation: categorical- anuric vs uric (threshold=200mL/d)
e) Dialysis vintage: continuous
f) Diabetic Mellitus: Categorical- Y/N
g) Access Type: Categorical- AVF / Tunnelled haemodialysis line (THL)
h) Cause of ESRF: Categorical- Unknown, Chronic glomerulonephritis (GN), Tubulo interstital nephritis (TIN) /chronic pyelonephritis, cancer/trauma, congenital/familial, polycystic kidney disease, hypertension, diabetes mellitus and others

Treatment parameters:

a) ESA (weekly dose) (mean of the 2 run-in values)
b) Last dialysis checked dialysis adequacy (kt/v) : continuous
c) Prescribed hours of dialysis
d) Prescribed angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB): categorical – Y/N

Biochemical parameters (last available result prior to randomisation unless specified):

a) Haemoglobin (mean of the 2 run-in period values)

b) Hematocrit

c) Transferrin saturation

d) Serum Ferritin

e) Vitamin B12 level

f) Folate level

g) C- reactive protein

h) Parathyroid hormone

i) Corrected calcium

j) Phosphate

k) Total alkaline phosphatise

l) Pre-dialysis urea

m) Pre-dialysis creatinine

n) Pre-dialysis potassium

o) Albumin

p) kt/v

q) Urea reduction ratio (URR)

Associated primary outcome measures:

Haemoglobin, ESA dose and ERI (ratio for weekly ESA dose corrected for weight divided by Hb) were also analysed as associated primary outcome measures.

10.5 Cardiac magnetic resonance imaging scan protocol

All cardiac magnetic resonance (CMR) scans were undertaken on a Philips Achieva 1.5 T scanner (Best, Netherlands). All scans were ECG gated and performed with a dedicated 32-channel cardiac coil (Philips) using standardised protocols.

From coronal, sagittal and axial scout images, the short axis plane of the LV was identified and steady state free precision (SSFP) movie images were acquired

covering the entire LV from base to apex. There were at least 24 phases acquired per cardiac cycle.

Slice thickness was 6 millimeter (mm), with a 4mm gap between slices. Parallel imaging technology was utilised to ensure a reasonable breath-hold time (around 10-15s per slice). 10-12 slices were required to cover the LV. An axial plane was acquired at the level of the pulmonary artery bifurcation. High definition phase contrast imaging (50 phases per cardiac cycle) was performed over the ascending aorta and the descending thoracic aorta at this level. A further true axial plane was acquired in the mid abdominal aorta and phase contrast images were also acquired at this level.

CMR analysis

All data was anonymised and analysed by a single, experienced operator who was blinded to the patient information using CVi42 analysis software (Circle, Calgary). The end diastolic and end systolic frames of the short axis movie stack were identified. Manual contours were drawn around the epicardial surface of all the end diastolic frames and the endocardial surface of all end diastolic and end systolic frames. The basal slice was defined as the slice with at least 50% circumferential myocardial coverage.

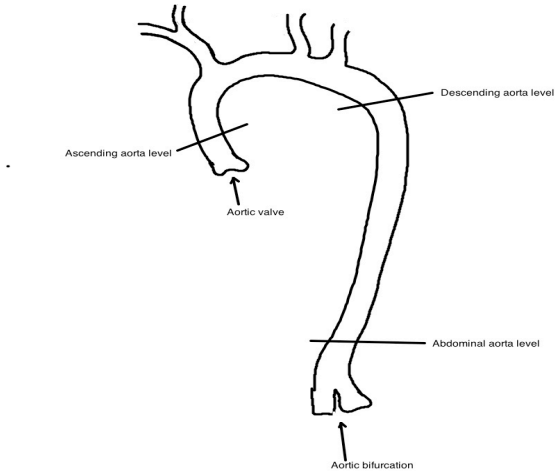
Papillary muscles were included in the blood pool and excluded from the myocardium. From these areas, the analysis software automatically calculated Left ventricular end diastolic volume (LVEDV), Left ventricular end systolic volume (LVESV), Left ventricular systolic volume (LVSV), Left ventricular ejection fraction (LVEF) and Left ventricular (LV) mass.

Contours were drawn around the aortic region of the phase contrast images at ascending thoracic aorta, descending thoracic aorta and abdominal aorta levels (Figure 10.1). The time delay between end-diastole (the beginning of the QRS complex on the ECG) and the peak aortic flow at all 3 levels were calculated automatically. End diastolic and end systolic aortic areas at each level were also

derived, to give a measure of aortic compliance. Aortic compliance was calculated as fractional change in aortic size at an area divided by pulse pressure multiplied to minimum aortic size. Aortic compliance value at ascending aorta was used in analysis.

The perpendicular distance between the thoracic descending aorta and the abdominal aorta was measured using coronal scout images. The time difference between the 2 respective flow peaks divided by the distance equaled blood flow velocity in second/centimetres . 20 randomly assigned scans were re analysed by the same operator to determine the intra-observer reproducibility.

Figure 10.1. Aortic pulse velocity measurement levels



10.6 FDG PET CT scan protocol

All PET acquisitions were accompanied by a low-dose CT procedure to allow for attenuation correction and co-localisation of PET data. The parameters of these CT acquisitions had estimated effective dose of no more than 5 millisievert (mSv) each.

As plasma clearance in these patients is delayed, dynamic imaging from 0 to 60 minutes over the area starting from the Arch of the Aorta (upper limit: arch of the aorta and lower limit: the axial extent of PET Field-of-View) was performed followed by whole body imaging at 90 minutes during initial study period.

Patients undergoing FDG-PET CT scan had blood glucose measurement carried out prior to administration of ^{18}FDG. All patients were fasted for 6 hours before imaging. Patients with blood sugars >10 mmol prior to imaging were excluded from PET CT imaging. The administered activity of ^{18}F-FDG was < 200 megabecquerel (MBq) due to renal failure in these patients. 200MBq was not be exceeded. Helical CT acquisition for the low-dose CT was from the base of the skull to the knee joints.

Whole Body PET data: Multiple bed positions were acquired from the knee joints to the base of the skull. The time per bed position was 2 minutes and the PET data acquisition did not exceed a total of 30 minutes. Dynamic scan started at injection time (20 seconds delay); followed by whole body scan at 90 minutes from injection time. No more than 1 x 60 minutes and 1 x 35 minutes (max. 30 min PET emission). Blood sampling: 3 samples (3mls each) at minutes 1, 2 and 3 form the injection of activity; then 3 samples (3mls each) at minutes 5, 7 and 9 followed by 5 samples at 19, 29, 39, 49 and 59 minutes) (total 11 samples =33 mls blood sample per scan). Dynamic imaging protocol was stopped after first 10 dynamic PET CT tests. Subsequently scan data was acquired at 90 minutes following ^{18}F-FDG administration.

Image analysis

Image analysis were performed on a dedicated workstation. Using the CT images, the vasculature was divided into carotid arteries, the aorta, iliac and femoral arteries. The common and external iliac arteries were combined and treated together as ‘‘iliac artery’’; similarly, the common femoral and superficial femoral arteries were amalgamated into the single label of ‘‘femoral artery.’’ The transition point between iliac and femoral arteries was inguinal ligament. Arterial 18F-FDG uptake (as a measure of arterial inflammation) was measured by drawing a region of interest (ROI) around the artery on every slice of the co registered trans axial PET/CT images. On each image slice, the mean and maximum standardized uptake values (SUVs) of 18F-FDG in the ROI (containing the arterial wall and the lumen) was calculated as the mean and maximum pixel activity. The SUV is the decay-corrected tissue concentration of 18F-FDG (in kBq/g), adjusted for injected 18F-FDG dose and body weight (in kBq/g), and is a well-recognized method for quantification of 18F-FDG PET data.

By averaging SUVs for all artery slices within an arterial territory, mean and maximum SUVs can be derived for each region. These SUVs are normalized to blood 18F-FDG activity by division by an average blood ROI (at least 8 venous ROI measurements), estimated from either the inferior vena cava (leg studies) or the superior vena cava. This calculation results in an arterial TBR measure, which was reported subsequently. Figure 10.2 demonstrates an example of calculation of TBR along the ascending aorta section [95]. Similarly, this calculation was done along all artery sections described above.

Figure 10.2: Calculation of Target to Background ratio (TBR) [95]

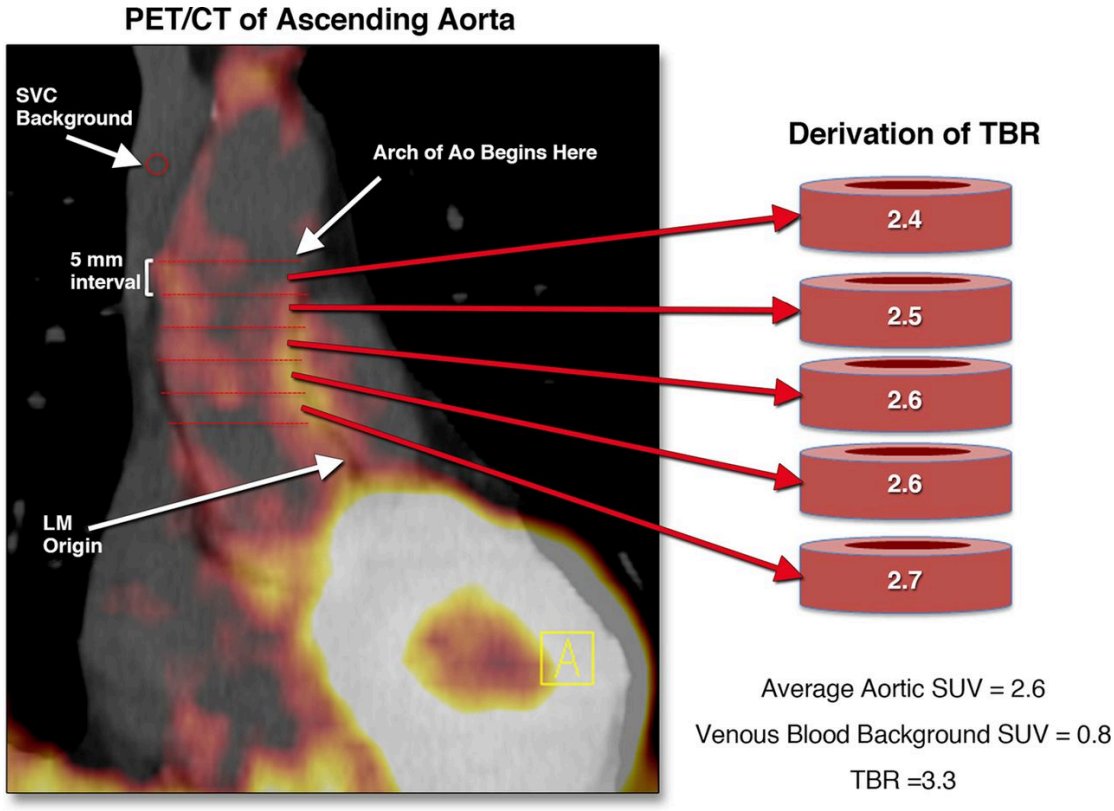


Figure 10.2

Example for calculation of the target to background ratio (TBR) along section of ascending aorta. Axial sections were taken at approximately 5 mm thickness. Subsequently region of interest (ROI) was drawn around the wall of the artery. Maximum standardised uptake value for FDG uptake was recorded as SUVmax for each axial section (depicted on rings next to arrows). Venous blood SUVmax was obtained from superior vena cava (SVC) over 10 sections (ROIs) and averaged to get blood compartment contribution. TBR was obtained by dividing mean SUV max from axial slices with SUVmax obtained from SVC. (Ao = aorta; LM = left main artery; SVC = superior vena cava)

10.7 Specimen Analysis

Haematology

Haematological indices were measured routinely in the Haematology laboratories of the Royal London Hospital on a Sysmex XE-2100 Haematology Analyser (Syzmex Uk Ltd, Milton Keynes, UK).

Biochemistry

All biochemistry analysis was performed in the Biochemistry Laboratories of the Royal London Hospital on a Roche Modular E019 P-Unit Analyser or a Roche Modular E170 Analytics EVO Analyser (Roche Diagnostics Ltd, Burgess Hill, UK). Serum and urine creatinine was measured by a kinetic colorimetric assay modified from the Jaffe reaction described in 1886 and standardised in 1945. Urine protein was measured using a turbidometric assay.

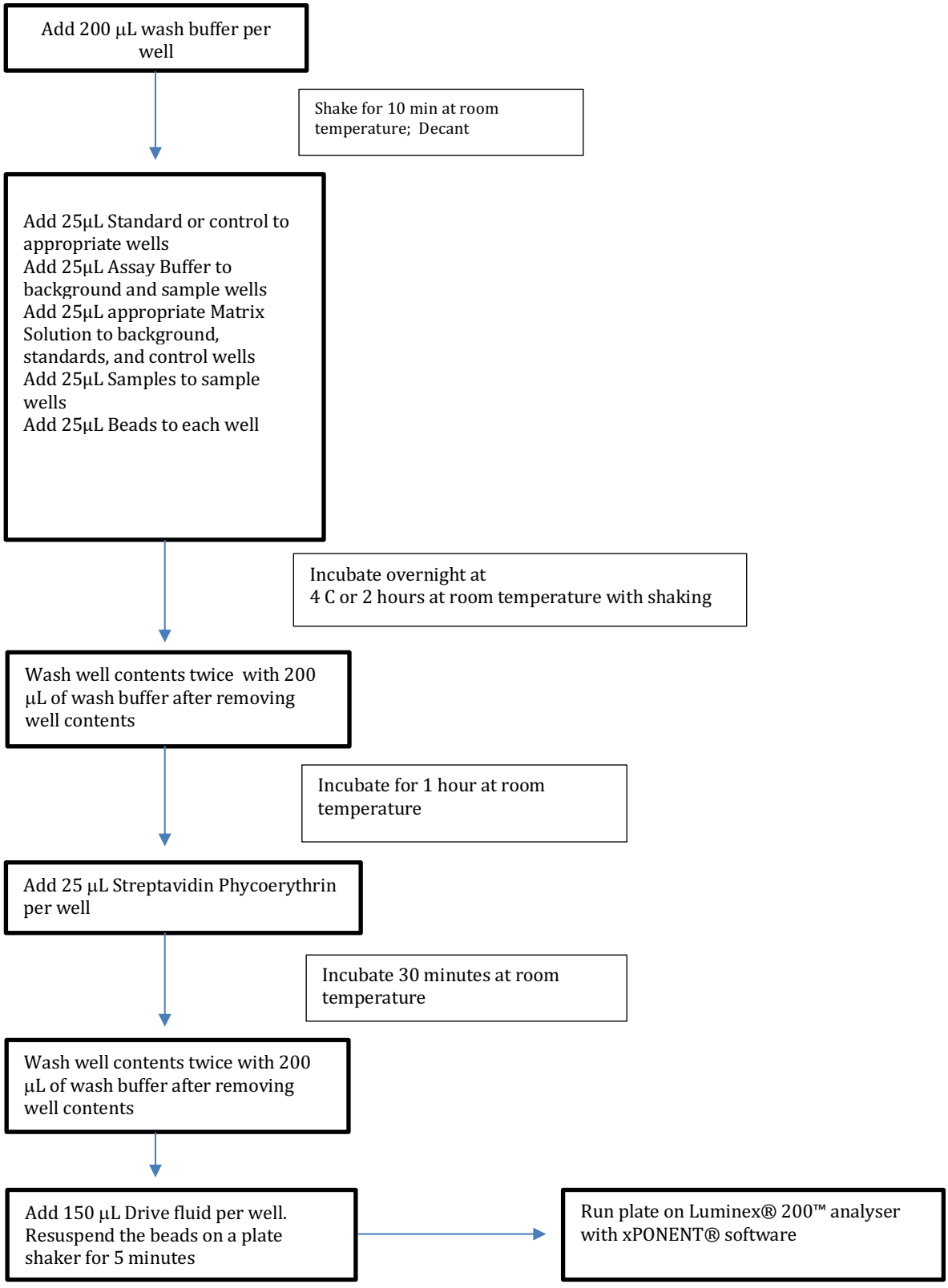
Mechanistic end points: Cytokine analysis

EMD Millipore's MILLIPLEX® MAP Human Cytokine / Chemokine Panel which is based upon Luminex xMAP technology was used for the simultaneous quantification of cytokines using pre-mixed Magnetic Beads for selected cytokines. The xMAP technology analyses reactions over 3 dimensional structures of magnetic microspheres when passed through a flow analyser armed with two lasers. Subsequently high speed digital processing and computer software convert florescent information from magnetic beads into numerical results.

Reagents supplied with cytokine analysis kit were Human cytokine / chemokine standard, Human cytokine quality controls 1 and 2, Serum Matrix (0.08% Sodium Azide), Wash Buffer (0.05% Procyclin), Human cytokine detection antibodies, Streptavidin-Phycoerythrin and Premixed human cytokine / chemokine antibody immobilised magnetic beads.

Frozen serum sample was mixed by vortexing and centrifuge after thawing completely to room temperature. 96 well plate was used for assay. Standards, controls and samples were placed in a vertical configuration. Plates were run on Luminex® 200™ analyser with xPONENT® software. Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating cytokine concentrations in samples was obtained. Two assays were done for each sample which were subsequently compared for quality control. Detailed analysis protocol is demonstrated in figure 10.3.

Figure 10.3: Cytokine analysis protocol



10.8 Summary of study design

Figure 10.4 describes study design. Blood investigations were done at every visit as per the study protocol. Additional blood samples were taken during each study visit for future research. These samples were stored at -80 C temperature. Subsequently these stored samples were analysed for cytokine levels during the course of study.

Initially end point for analysis of investigation results and ESA dose was planned to be the mean of values at visit 10 (week 26) & 11 (week 28) during the follow up phase. However, thesis examiners suggested to reanalyse end point data at visit 9 (week 24) to avoid any drug washout effect during the follow up phase. Hence results given below are after reanalysis of relevant data at visit 9 (week 24).

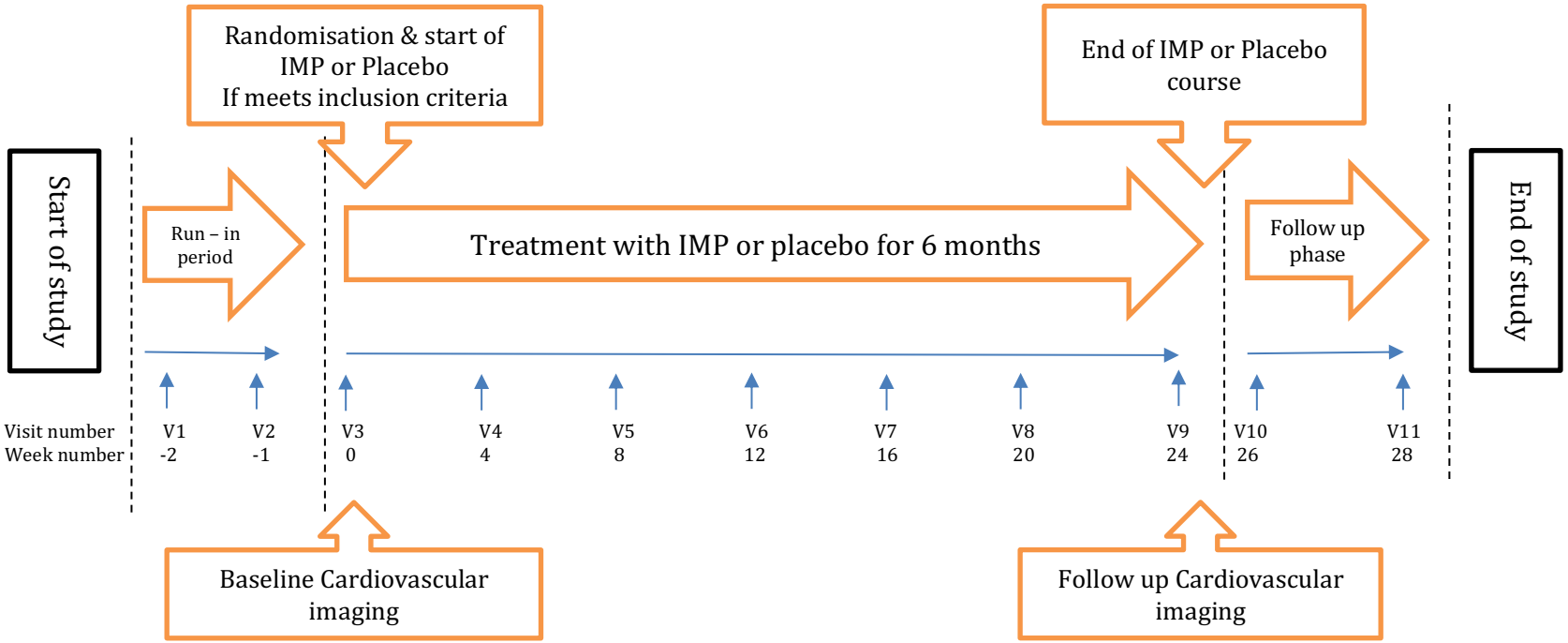


Figure 10.4. Summary of study design.
Investigations and other study parameters were recorded on every visit (V1 to V11)

10.9 Amendments to study inclusion criteria

Along with the newer emerging trends and guidance in the field of ESA hyporesponsiveness research in CKD population, we also came across practical challenges during early recruitment phase. Hence inclusion criteria defining ESA hyporesponsiveness was revised. This was also done for the study results to remain relevant for planning further research based on the trends of other research studies published at that time. Substantial amendments made to inclusion criteria, after the start of patient recruitment are summarised below:

10.9.1 Definition of ESA hyporesponsiveness and change to inclusion criteria

At present there is no widely accepted guideline to define ESA hyporesponsiveness world-wide. This is reflected by variation among international guidelines. K/DOQI guidelines define ESA Hyporesponsiveness as weekly ESA requirement greater or equal to 450 units/kg or 300 units/kg for intravenous and subcutaneous administration respectively[118].

European guidelines define ESA hyporesponsive state as weekly ESA requirement of 300 units/Kg for recombinant erythropoietin (EPO) or 1.5 mcg/kg for Darbepoeitin treated patients[119].

On the other hand, KDIGO guidelines do not provide any absolute cut off dose to define ESA hypo responsiveness. KDIGO defines initial ESA hyporesponsiveness as no increase in haemoglobin concentration after first month of appropriate weight-based ESA dosing. Acquired hyporesponsiveness is defined as requirement of 2 increments in ESA dosing up to 50% beyond the dose at which Haemoglobin had been stable originally[120].

Multiple clinical trials investigating ESA hyporesponsiveness have considered definition of ESA hyporesponsiveness based on the ESA dose trends in the local cohort of patients. The RISCAVID study classified patients with erythropoietin resistance index (ERI) values falling into fourth quartile (>15.4 IU weekly dose divided by weight and Hb value) as ESA hypo responsive in a cohort of 757 dialysis patients with dialysis vintage of more than 90 days[121].

Slow recruitment in a trial investigating ESA hyporesponsiveness resulted in change in inclusion criteria from an initial definition of Hb concentration less than or equal to 110 g/l for at least 3 months despite erythropoietin dose of greater or equal to 200 IU / Kg / week or darbepoetin dose greater or equal to 1mcg/kg/week for atleast 1 month to Hb concentration less than or equal to 120 g/l and ERI value greater or equal to 1 IU / Kg / week /g/L or 1mcg/kg/wk/ g / L for darbepoetin treated patients[122, 123].

We also anticipated that PEAR study recruitment may be slowed down with our initial inclusion criteria. Therefore, in the light of emerging trends for the definition of ESA resistance in multiple trials, the inclusion criteria for PEAR study was also amended from

"Receiving treatment with IV or SC erythropoietin receptor agonist at least weekly (ie exclude Micera or other ESAs given fortnightly or monthly) for a minimum of 8 weeks prior to administration of study agent, requiring doses to remedy EPO resistance (requiring greater than or equal to 12,000 iu equivalent of EPO per week or if ESA dose is greater than or equal to 150 iu equivalent of EPO/kg body weight/week), with evidence of stable haemoglobin levels"

to a revised criterion of

"Receiving treatment with IV or SC erythropoietin receptor agonist at least weekly (ie exclude Micera or other ESAs given fortnightly or monthly) for a minimum of 8 weeks prior to administration of study agent, requiring doses to remedy EPO-resistance (requiring greater than or equal to 6000 iu equivalent of EPO per week or if ESA resistance index is greater than or equal to 6.5 iu /kg/wk/g Hb for equivalent EPO dose), with evidence of stable haemoglobin levels"

We chose our new threshold because internal audit data showed that our proposed threshold represents the median ESA requirement in all the haemodialysis patients with arteriovenous fistula in our unit. ESA resistance index (ERI) is a better indicator of ESA dose relative to haemoglobin level as it gives exact ESA load for the individual patient to achieve per gram of haemoglobin. We therefore included ERI in the new inclusion criteria to define ESA hyporesponsiveness.

10.9.2 Removal of non-highly sensitive C reactive protein from the inclusion criteria

Unfortunately, the central lab at The Barts Health NHS Trust did not routinely measured non-highly sensitive CRP. There is evidence to suggest non-highly sensitive CRP is not a good marker of inflammation in haemodialysis. New literature had identified other biomarkers such as hepcidin and pro inflammatory cytokines to correlate with ESA hypo responsiveness (but not non highly sensitive CRP)[124, 125].

Therefore, using CRP >5 as an inclusion criterion would have resulted in under representation of the ESA hypo responsiveness secondary to inflammation in our cohort of haemodialysis patients).

11. Results

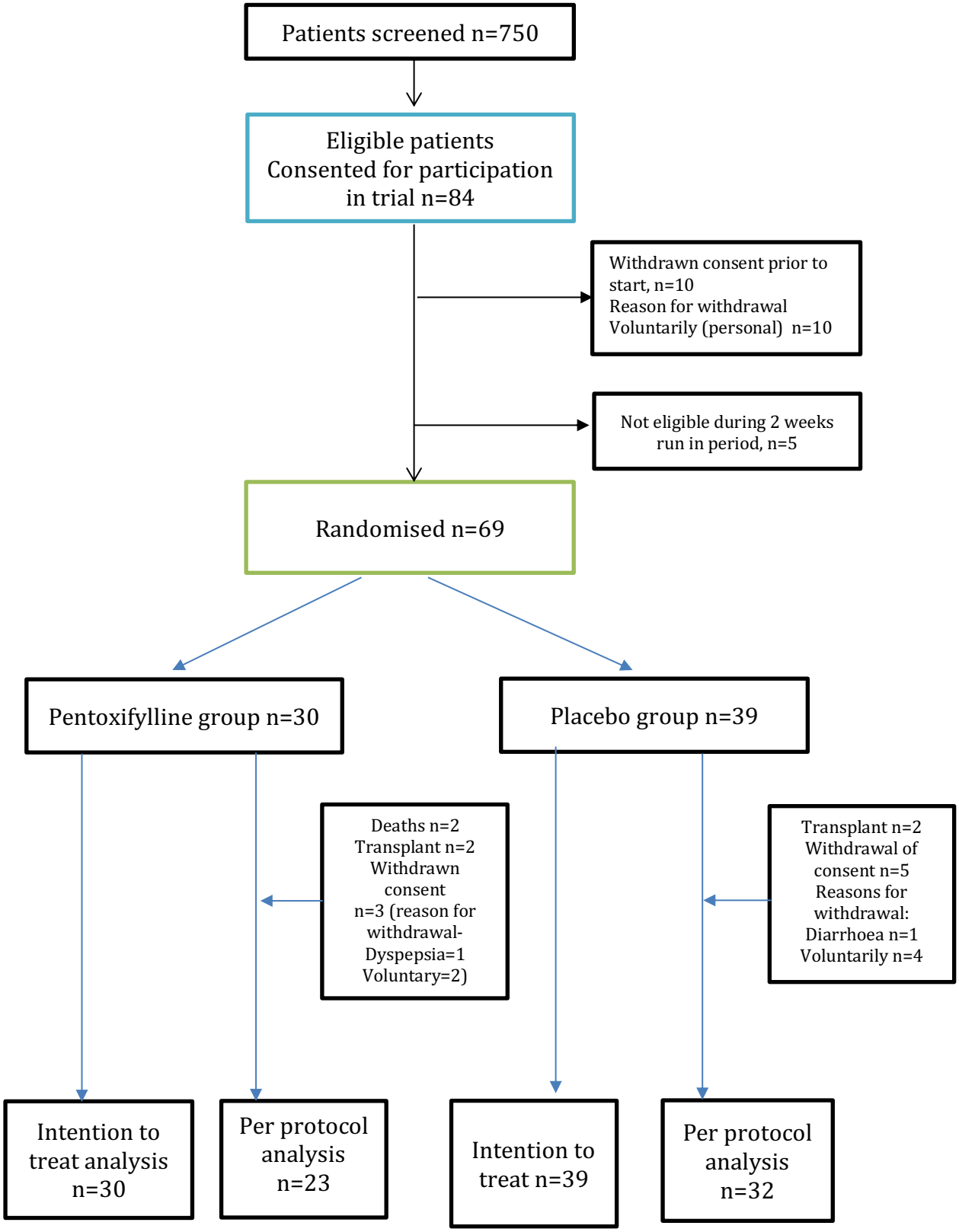
11.1 Patient allocation

A total of 750 patients were screened for ESA hyporesponsiveness. 84 eligible patients consented to participate in the study after screening of haemodialysis database on regular intervals. 10 patients withdrew consent voluntarily prior to start of study protocol. Further 5 patients were found to be not eligible for study during 2 weeks of run in period prior to randomisation.
69 patients entered randomisation phase. 30 patients were allocated to Pentoxifylline group while 39 patients were allocated to placebo group.

In the Pentoxifylline group, 2 patients were transplanted during the course of study and 1 patient withdrew consent due to dyspepsia symptoms. 2 patients died in Pentoxifylline group.

In placebo group, 2 patients received kidney transplant and 5 patients withdrew consent from the study. 4 patients withdrew consent voluntarily due to personal reasons while 1 patient withdrew consent due to gastro intestinal side effects. All 39 patients were included in ITT analysis (Figure 11.1).

Figure 11. 1 Patient allocation during study



11.2 Baseline characteristics

The baseline characteristics of patients in Pentoxifylline and placebo groups did not show any statistically significant difference except for haemoglobin and corrected calcium levels (Tables 11.1 a & b). All patients were dialysing using polysulfone dialyser membranes. All patients had urine output less than 200mls in 24 hrs.

Table 11.1 a Baseline characteristics- demographics

	Pentoxyfylline	Control	*p* value
Age (years)*	56.21 (13.53)	56.05 (14.06)	0.96
Gender (male)	24 (85%)	29 (74%)	0.29
Ethinicity Asians (Indian subcontinent) Black White Others	 10 (35%) 11 (39%) 7 (25%)	 13 (33%) 14 (35%) 10 (25%) 2 (5%)	 0.84 0.77 0.95
Cause of CKD Diabetes Mellitus Hypertension Chronic Glomerulonephritis Adult Polycystic Kidney disease Tubulo interstitial nephritis Congenital Others Unknown	 13 (46%) 06 (21%) 03 (10%) 3 (10%) 3 (10%)	 16 (41%) 06 (15%) 08 (20%) 02 (05%) 1 (2%) 2 (5%) 4 (10%)	 0.65 0.63 0.28 0.38 0.38
Diabetes Mellitus	13 (46%)	21 (53%)	0.54
Prescribed ACE inh or ARB	12 (42%)	14 (35%)	0.56
Dialysis access type- **Arterio venous fistula**	17 (60%)	25 (64%)	0.77
Dialysis vintage (months) **	22.5 (8-44.5)	20 (12-60)	0.49
Prescribed duration of dialysis per week (hours)*	11.79 (0.88)	11.88 (0.88)	0.65
Dialysis adequacy (kt/v) *	1.48 (0.24)	1.60 (0.38)	0.12

*The categorical values are given as numbers (percentages) while continuous variables are given as mean (standard deviation) * or median (interquartile range) **.*

Table 11.1 b. Baseline characteristics – laboratory parameters

	Pentoxifylline	Control	*p* value
ESA/Hb ratio (mean of 2 run-in values) **	3.02 (2.51-5.30)	3.92 (2.76-6.56)	0.06
ESA/Hb/weight ratio (mean of 2 run-in values) **	0.10 (0.06-0.13)	0.12 (0.07-0.15)	0.27
Haemoglobin (Hb) g/dl (mean of 2 run-in values) *	11.34 (0.89)	10.74 (0.92)	0.01
Haematocrit *	0.33 (0.03)	0.32 (0.02)	0.10
Transferrin saturation **	26 (22-34)	24 (20-29)	0.12
Ferritin mcg/L*	415.8 (235.2)	461.6 (174.7)	0.51
C reactive protein mg/L (non-highly sensitive) **	6 (5-14.7)	7 (5- 16)	0.72
Parathyroid hormone pmol/L **	33.05 (19.3- 56.6)	25.6 (14.8 – 52.8)	0.29
Corrected Calcium mmol/L **	2.23 (2.12-2.31)	2.3 (2.24-2.44)	0.02
Phosphate mmol/L*	1.79 (0.54)	1.74 (0.66)	0.77
Total alkaline phosphatase IU/L *	87.5 (62.25-128.3)	91 (64-101)	0.79
Pre-dialysis urea mmol/L *	20.72 (5.60)	19.76 (4.82)	0.45
Pre-dialysis creatinine µmol/L **	915 (695.8-1184)	836 (698-979)	0.30
Pre-dialysis potassium mmol/L *	5.02 (0.53)	5.01 (0.72)	0.93
Albumin g/L	41.59 (3.60)	41.1 (3.87)	0.60

*The categorical values are given as numbers (percentages) while continuous variables are given as mean (standard deviation) * or median (interquartile range) **. Abbreviation: Erythropoiesis stimulating agent, ESA.*

11.3 Primary and associated outcome measures

Per protocol analysis:
The primary outcome of ESA dose in relation to Hb (ESA/Hb ratio) showed lower mean (SD) ESA/Hb ratio 4.08 (3.31) in Pentoxifylline group compared to Placebo group 4.67 (3.54) but it failed to reach statistical significance (p value = 0.53).

Intention to treat (ITT) analysis:
The primary outcome of ESA dose in relation to Hb (ESA/Hb ratio) showed lower ESA/Hb ratio in Pentoxifylline group compared to Placebo group but it also failed to reach statistical significance (p value = 0.12). Comparison within individual groups showed an improvement in Pentoxifylline group (mean difference= -0.349, p value = 0.62) compared to placebo (mean difference = 0.25, p value= 0.68). Associated primary outcomes including Hb, ESA dose and ERI also did not show any statistically significant difference between the groups.

Intra group analysis of associated outcomes showed an improvement in ESA/Hb ratio and ESA dose in Pentoxifylline group while Haemoglobin levels showed improvement in placebo group. None of these changes were of any statistical significance (Table 11.2 & Figures 11.2 a-d). Comparison of mean values at every study visit point did not show any significant difference between the groups did not show any significant difference for primary or any of the associated outcomes (Figures 11.3 a-d).

The results remain similar to previous analysis. No statistically significant different outcomes were noted even after change in data analysis end point from mean of results from two follow up study visits, 2 weeks and 4 weeks after the end of IMP intake period to the results from the visit immediately after the end of IMP intake period.

Table 11.2 Primary and associated outcomes (Intention to treat analysis)

ESA – erythropoietin stimulating agent (Darbepoetin dose in mcg), Hb- Haemoglobin (gm/dl), ERI- erythropoietin resistive index (ESA/ Hb / Weight)

Outcome	Placebo (n=39)			Pentoxifylline (n=30)			p value
	Baseline	Follow up	Mean difference	Baseline	Follow up	Mean difference	Follow up groups (placebo & pentoxifylline)
ESA /Hb	*4.77 (2.37)*	*5.03 (3.18)*	*-0.25*	*4.18 (2.30)*	*3.83 (2.91)*	*0.34*	*0.12*
Hb	10.74 (.92)	11.01 (1.30)	-0.27	11.34 (.89)	11.14 (1.09)	0.19	0.66
ESA	50.25 (23.94)	52.95 (32.84)	-2.69	44.29 (23.28)	42.32 (28.59)	1.96	0.15
ERI	.061 (.02)	.063 (.03)	0.001	.053 (.02)	.053 (.02)	-0.0009	0.25

1. *Means of first two ESA / Hb values during run-in period (baseline) and ESA / Hb values at the start of follow up phase*
2. *Mean difference = baseline value – follow up value in each cohort.*
3. *Results displayed as Mean (standard deviation)*

Figures 11.2 (a-d). Primary and associated outcomes (ITT analysis)

Figure 11.2 a. ESA/Hb ratio in placebo and Pentoxifylline groups

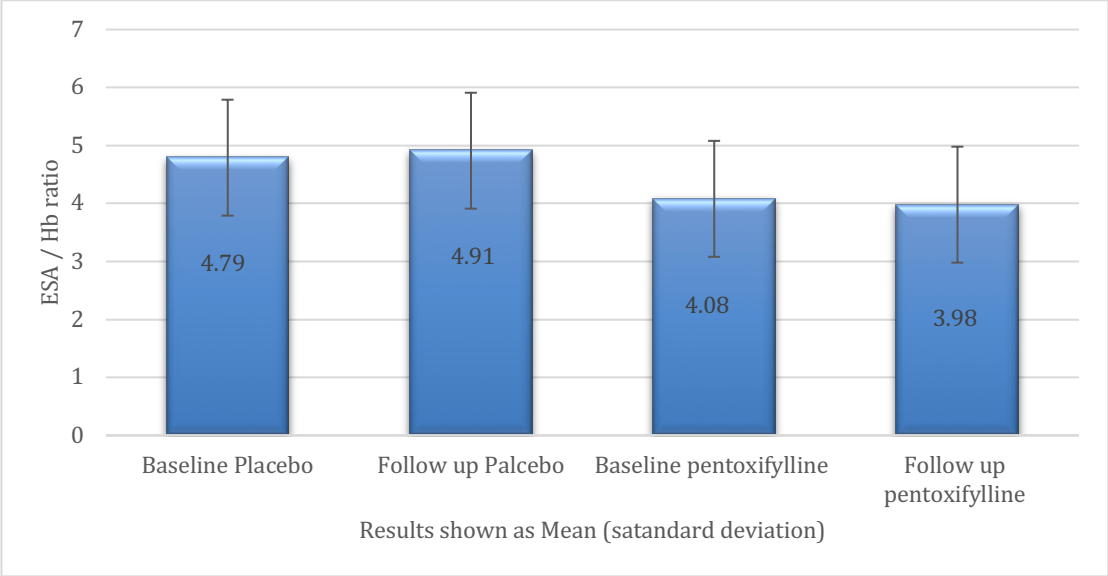


Figure 11.2 b. Haemoglobin in placebo and Pentoxifylline groups

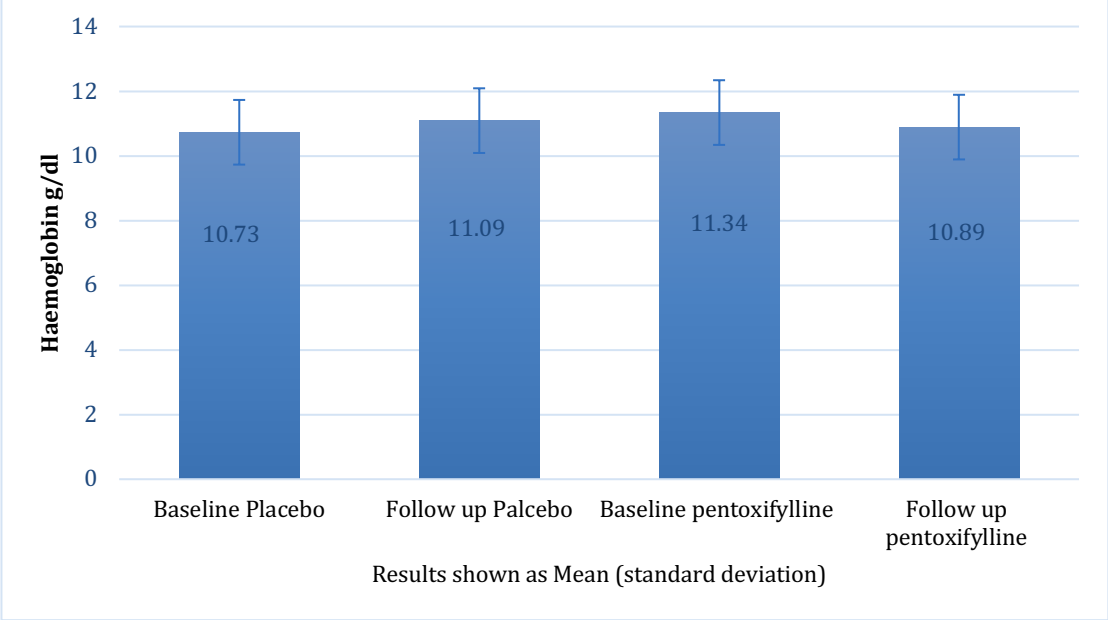


Figure 11.2 c. ESA dose in placebo and Pentoxifylline groups

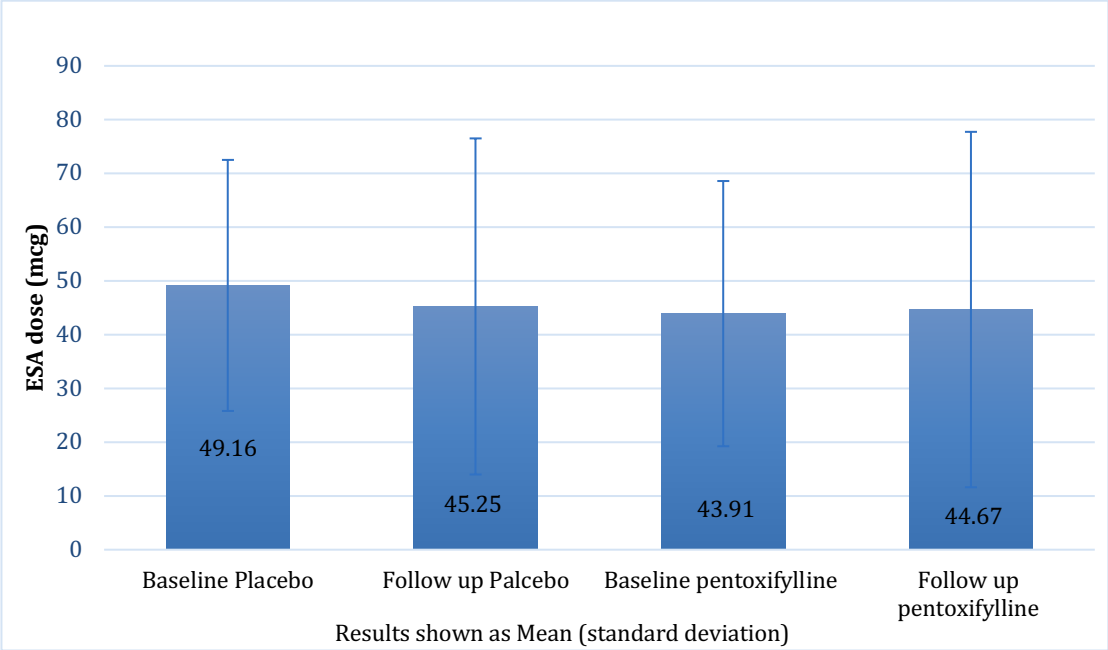
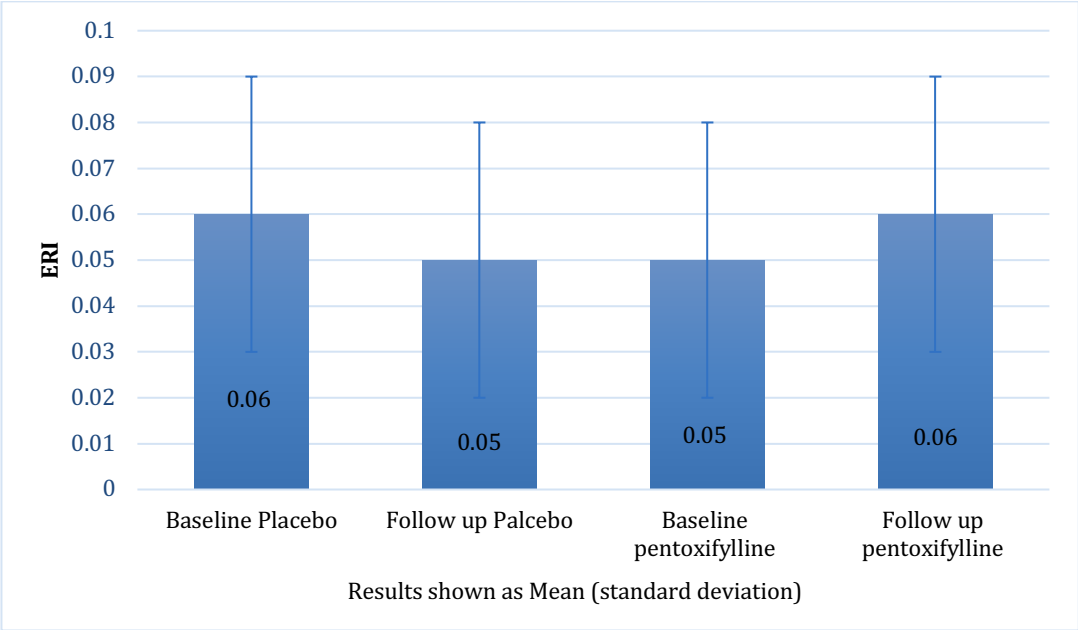


Figure 11.2 d. ERI in placebo and Pentoxifylline groups



11.3.1 Primary and associated outcomes measure variability during course of study

Figures 11.3 (a-d) Primary and associated outcomes measure variability during the course of study

Figure 11.3a Variability of mean ESA/ Hb ratio between groups during the course of study

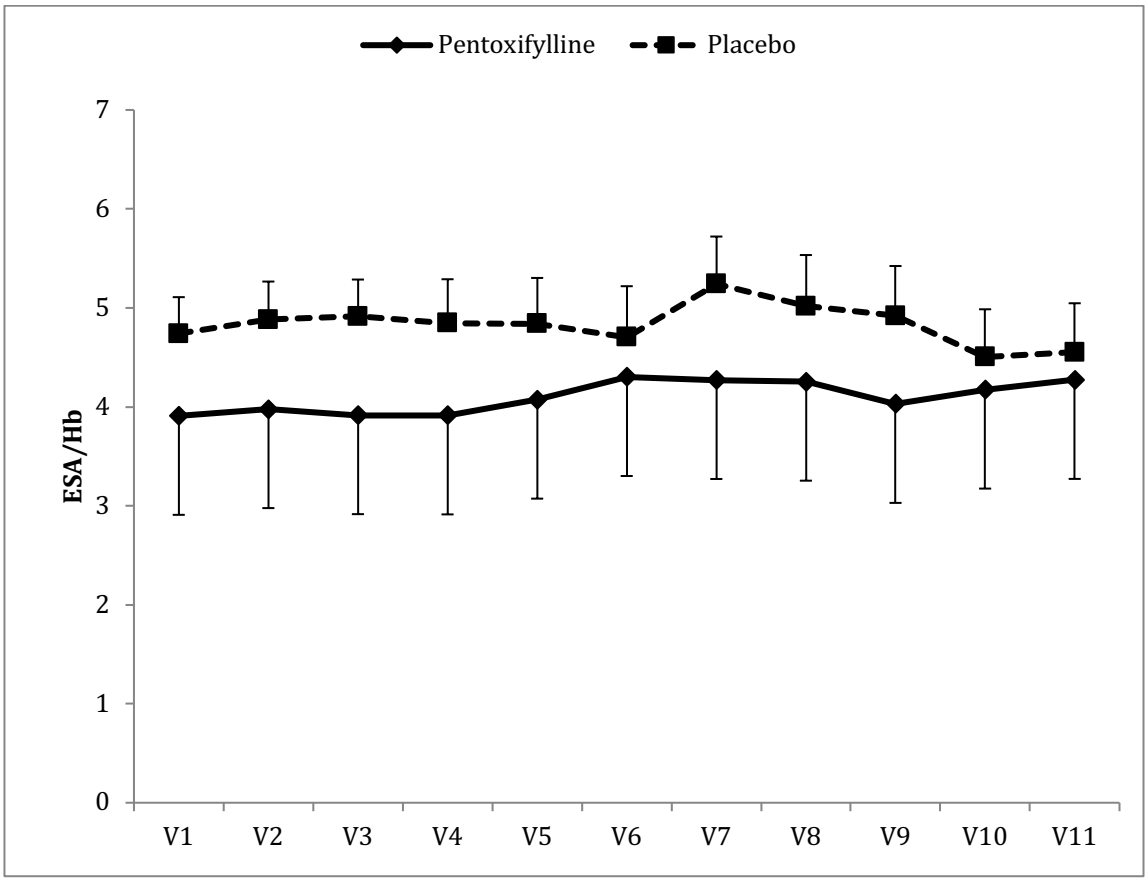


Figure 11.3a

Every study point depicts mean with standard deviation in both groups. Comparison of mean ESA/Hb between placebo and pentoxifylline group at every study visit along the course of study did not show any significant statistical difference.

Figure 11.3b Variability of mean ESA dose values between study groups during the course of study

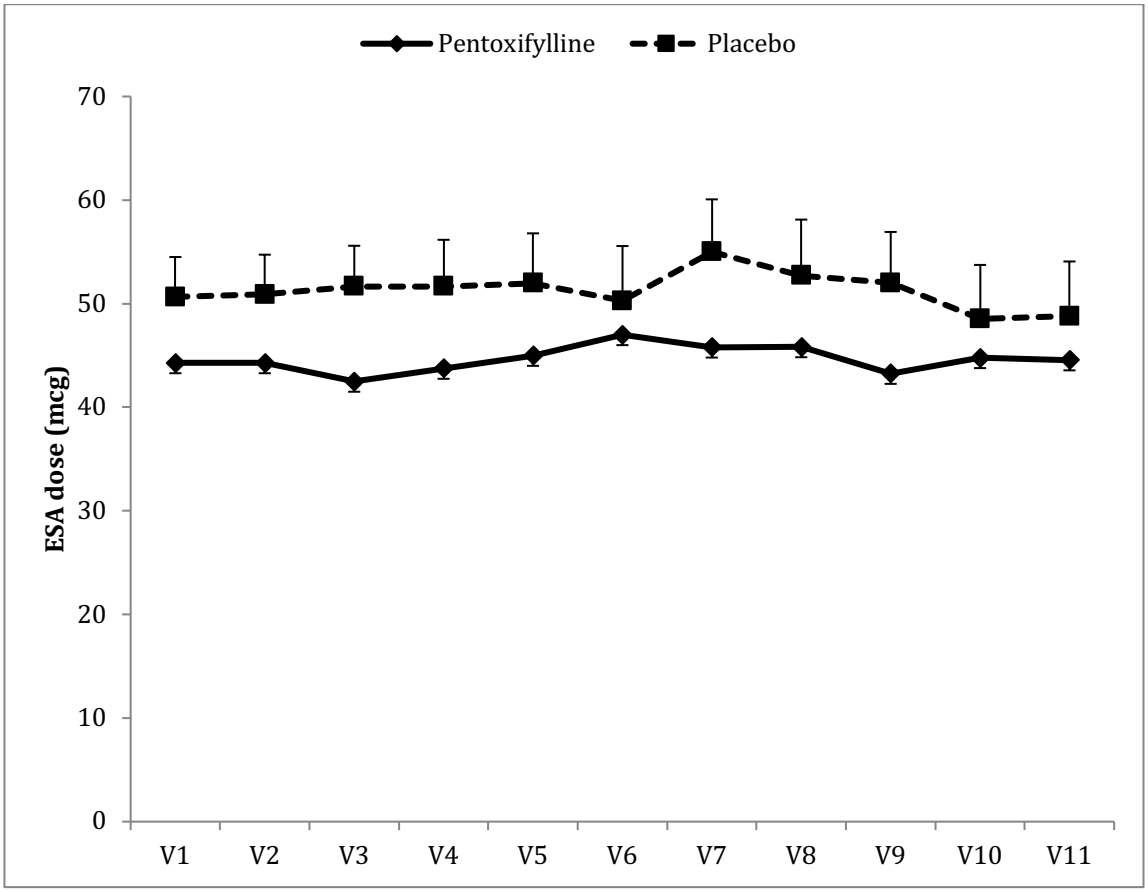


Figure 11.3b

Every study point depicts mean with standard deviation in both groups. Comparison of mean ESA dose between placebo and Pentoxifylline group at every study visit along the course of study did not show any significant statistical difference.

Figure 11.3c Variability of mean Haemoglobin between study groups during the course of study

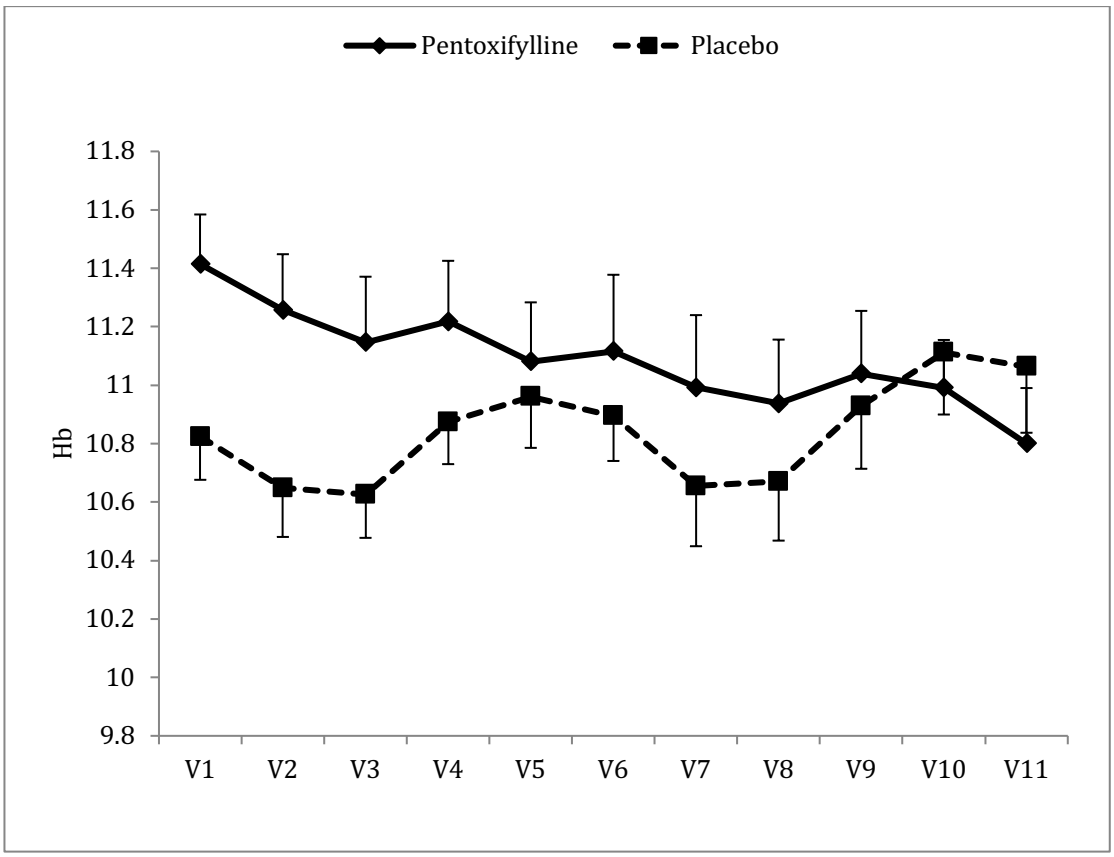


Figure 11.3c

Every study point depicts mean with standard deviation in both groups. Comparison of mean Haemoglobin between Placebo and Pentoxifylline group at every study visit along the course of study did not show any significant statistical difference after randomisation.

Figure 11.3d Variability of mean ERI between study groups during the course of study

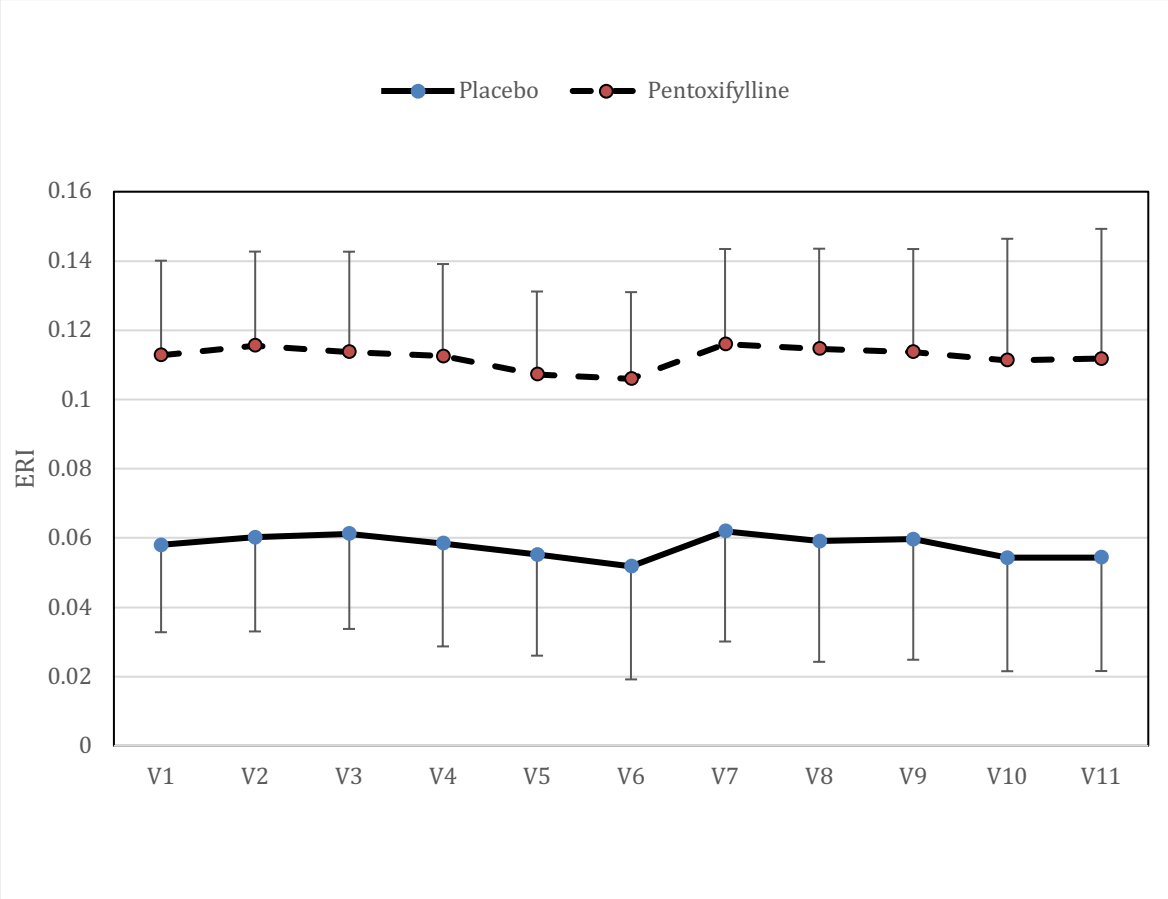


Figure 11.3d
Every study point depicts mean with standard deviation in both groups.

Comparison of mean ERI between Placebo and Pentoxifylline group at every study visit along the course of study did not show any significant statistical difference.

11.4 Secondary outcomes

11.4.1 PET CT Scan

Total 51 patients were able to attend optional baseline and follow up FDG PET CT scans. Cross sectional data analysis of SUV max and TBR across the whole cohort (n=51) at baseline showed mean (SD) of 2.60 (0.60) and 5.9 (2.93) respectively. 31 patients were in placebo group while 20 patients were in Pentoxifylline group. At baseline, there was no statistically significant difference in TBR or SUV max in Pentoxifylline and control group (Table 11.3a). On the follow up imaging at 6 months, there was no statistically significant difference in TBR or SUVmax in placebo or Pentoxifylline group (Figures 11.4 a & b). Similar trend was noted for percentage difference in TBR and SUV max with in Pentoxifylline group (Table 11.3b).

There was reduction in TBR with in the Pentoxifylline group but it failed to achieve statistical significance on the other hand there was a rise in TBR with in the placebo group at the end of study follow up period. There was reduction of SUV max with in both pentoxifylline and placebo group at the end of study follow up period (Figure 11.4 c & d).

Table 11. 3 a FDG PET CT Baseline characteristics

Outcome	Placebo (n=31)	Pentoxifylline (n=20)	p value
SUV max	2.55 (0.65)	2.68 (0.52)	0.44
TBR	5.88 (3.24)	5.82 (2.42)	0.94

Results displayed as mean (standard deviation); TBR- target to background ratio; SUV max – standardised uptake value maximum

Table 11.3 b FDG PET CT Results (Follow up results)

Outcome	Placebo (n = 31)	Pentoxifylline (n=20)	p value
SUV Max	2.53 (.47)	2.61 (.53)	0.64
TBR	6.04 (3.41)	5.27(1.94)	0.36
Difference SUV max (%)	8.53 (62.64)	-2.63	0.45
Difference TBR (%)	5.74 (22.95)	-1.05	0.39

Results displayed as mean (standard deviation)

Figures 11.4 18 FDG PET CT results

Figure 11.4 a. Target to background ratio (TBR) comparison at the end of the study period.

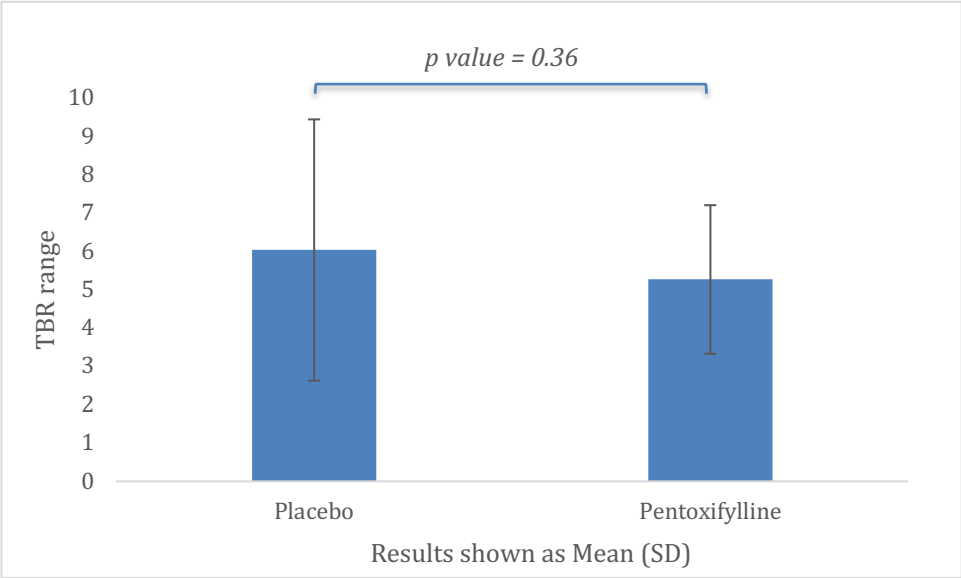


Figure 11.4 b. SUVmax comparison at the end of the study period

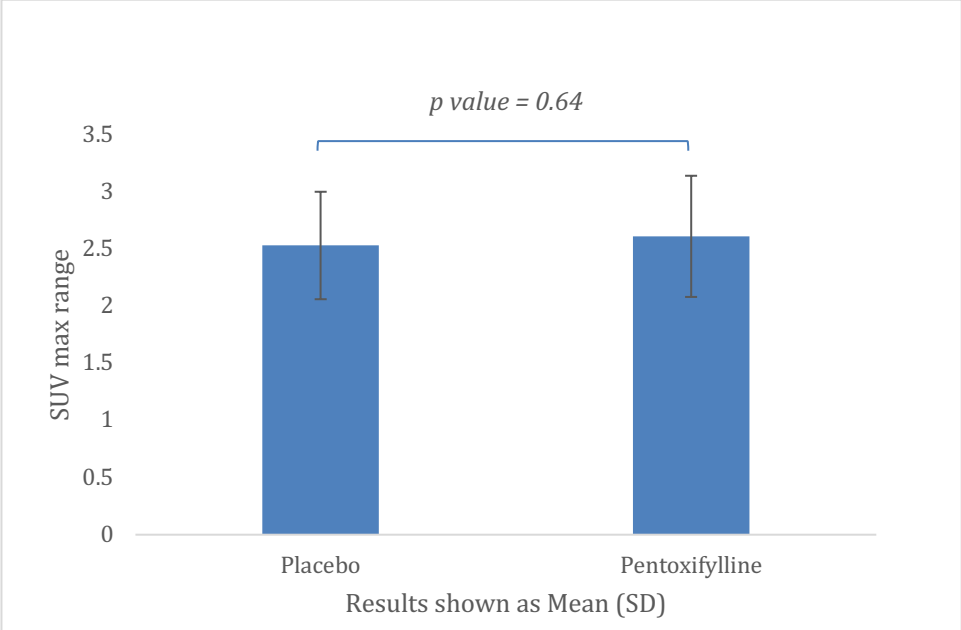


Figure 11.4 c. Target to background ratio (TBR) comparison with in the Placebo and Pentoxifylline groups

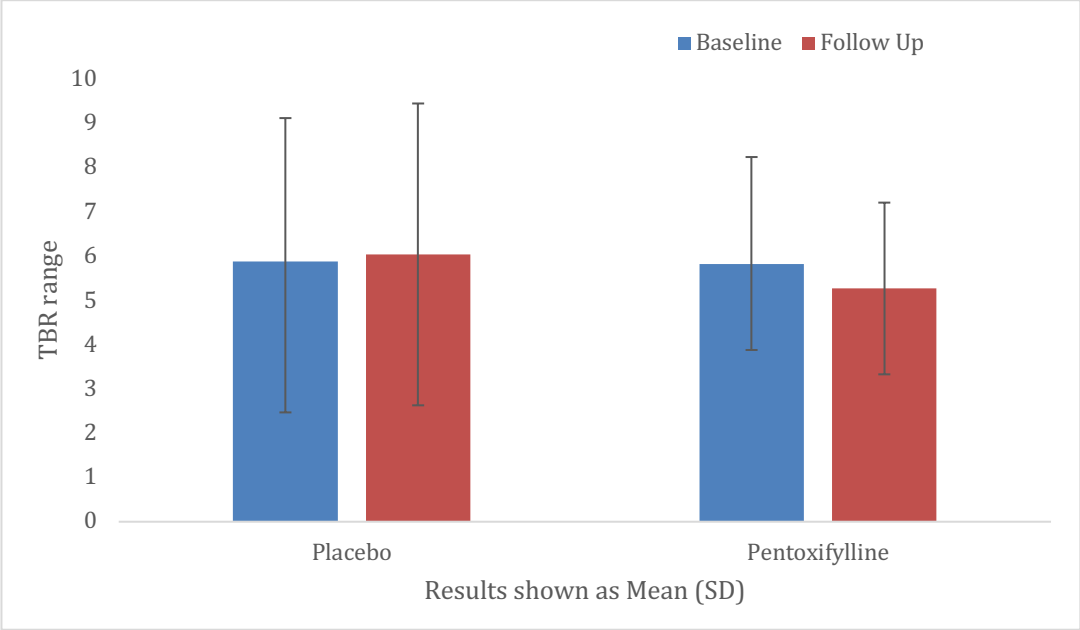
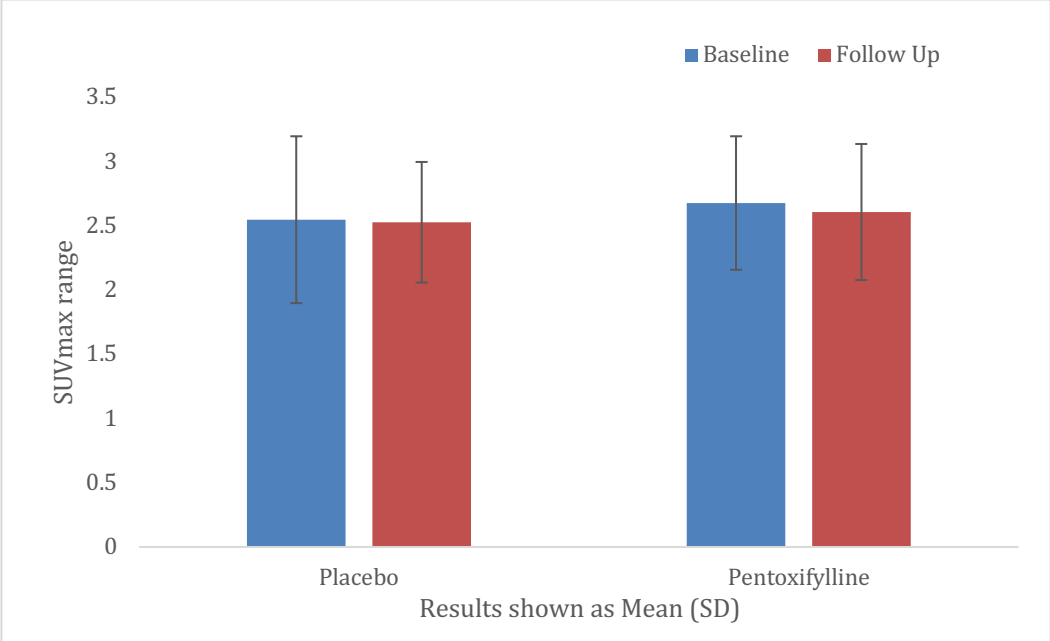


Figure 11.4 d SUVmax comparison with in the Placebo and Pentoxifylline groups



11.4.2 Cardiac MRI

In total, 29 patients in the study agreed to undergo the optional CMR scans. The baseline CMR results are shown in Table 11.4. There were no statistical differences in baseline CMR between the 12 patients who were randomised to pentoxifylline compared to the 17 patients randomised to control. After 6 months of treatment with either Pentoxifylline or Control, there were no statistically significant changes to the CMR parameters of End-Diastolic volume, End-Systolic volume, Stroke volume, Ejection Fraction or Myocardial Mass. There were also no inter-group (Pentoxifylline vs control) difference in the changes of these parameters that was statistically significant (Fig 11.5 a-e). However, there were significant reductions in aortic compliance in both groups.

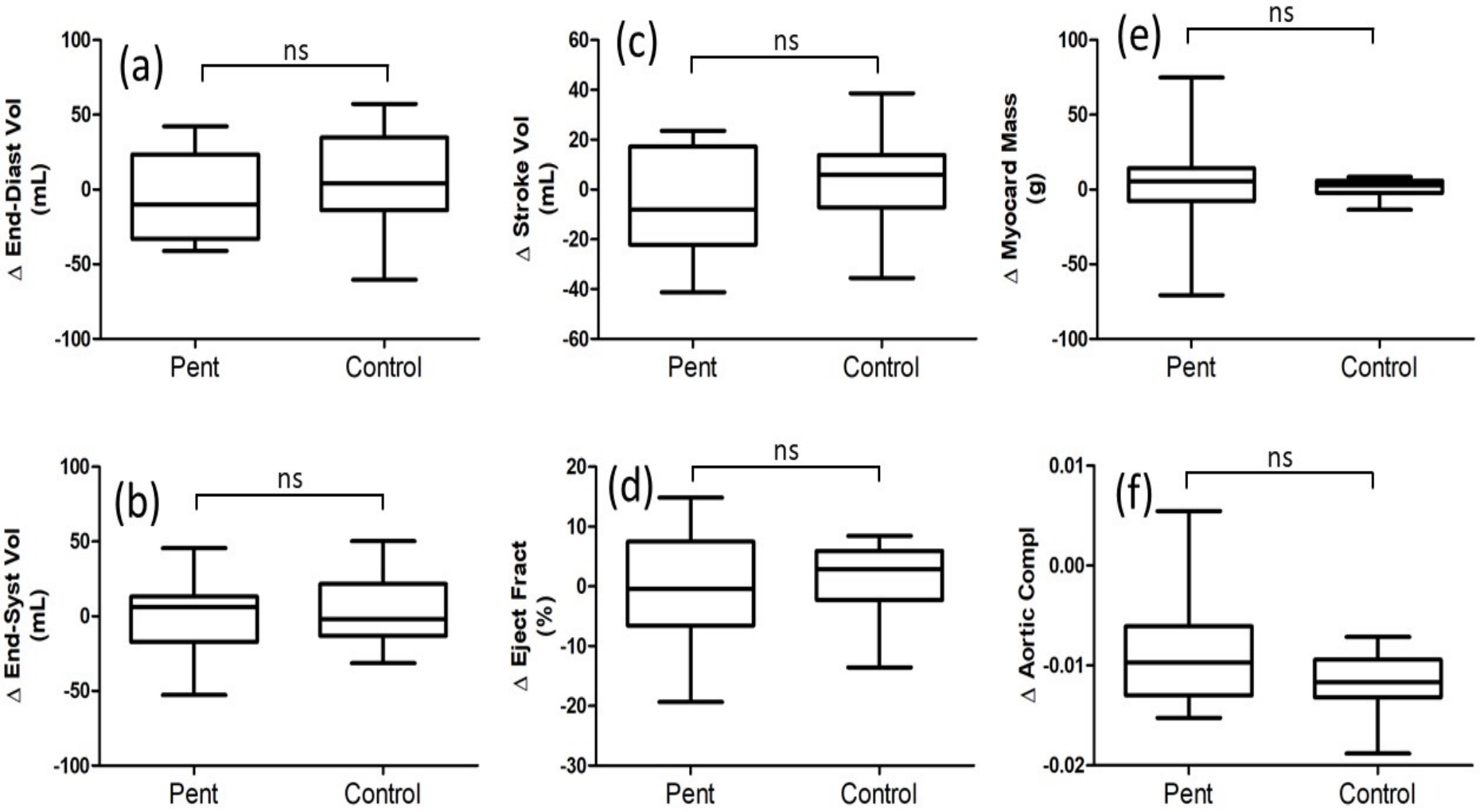
The aortic compliance of patients that received 6 months of Pentoxifylline decreased by -0.009 (95% CI: -0.005 to -0.012) units whilst the reduction for control patients was -0.012 (95% CI: -0.010 to -0.014) units. The difference in reduction between the Pentoxifylline vs Control patients were not statistically significant (Fig 11.5 f).

Table 11.4 Cardiac MR Baseline values

	Pentoxifylline	Control	P-value
Number	12	17	ns
End Diastolic Volume (mL)	177 (83.03)	150 (47.51)	ns
End Systolic Volume (mL)	78 (70.31)	63 (28.93)	ns
Stroke Volume (mL)	98 (33.69)	87 (24.94)	ns
Ejection Fraction (%)	59 (15.23)	59 (9.09)	ns
Myocardial Mass (g)	169 (77.74)	133 (36.84)	ns
Aortic Compliance (mm/Hg)	0.013 (0.003)	0.014 (0.004)	ns

Results displayed as mean (standard deviation); ns (p value not significant)

Figure 11.5 (a to f) Cardiac MRI results



11.5 Mechanistic endpoint: Cytokine analysis

Available serum samples from patients who had consented for their sample to be tested for experimental outcomes were analysed for cytokine profile during the course of trial. Comparison of cytokine titers at the end of the study period showed rise IL-6 , TGF beta and TNF titers in both pentoxifylline and control group except for statistically significant reduction IFN gamma titers when compared to placebo. There was reduction of IFN gamma titers with in the pentoxifylline group as well. (Table 11.5 and figures11.6 a-d).

On comparison of variability across Pentoxifylline and placebo groups during overall study period showed improvement in IL-6 and TNF levels in Pentoxifylline group with IL-6 levels achieving statistical significance. While the anti-inflammatory cytokines, TGF beta and IFN gamma levels were significantly higher in Pentoxifylline group compared to placebo group. (Figures 11.7 a-h).

Table 11.5 Cytokine analysis

Outcome	Placebo (n=39)			Pentoxifylline (n=30)			p value
	Baseline	Follow up	Mean difference	Baseline	Follow up	Mean difference	Follow up groups (placebo & pentoxifylline)
TNF alfa	24.81 (13.55)	29.17 (16.76)	-4.36	19.79 (14.11)	24.58 (17.05)	-4.78	.40
IL 6	9.28 (13.56)	15.57 (22.00)	-6.32	5.64 (7.47)	11.06 (11.06)	-5.42	.32
TGF	4.82 (4.95)	6.4 (5.2)	-1.64	6.59 (7.11)	7.61 (6.77)	-1.020	.34
IFN gamma	10.22 (17.27)	13.75 (18.48)	-3.52	5.68 (8.44)	4.32 (4.98)	1.35	.04

Means of first two readings and the last available reading during intervention phase (visit 9) were used for calculating baseline and follow up parameters respectively. Mean difference = baseline value – follow up value in each cohort.
Results displayed as Mean (standard deviation).
Cytokine titres were reported as picograms/millilitres

Figure 11.6 a. TNF alfa in placebo and pentoxifylline group

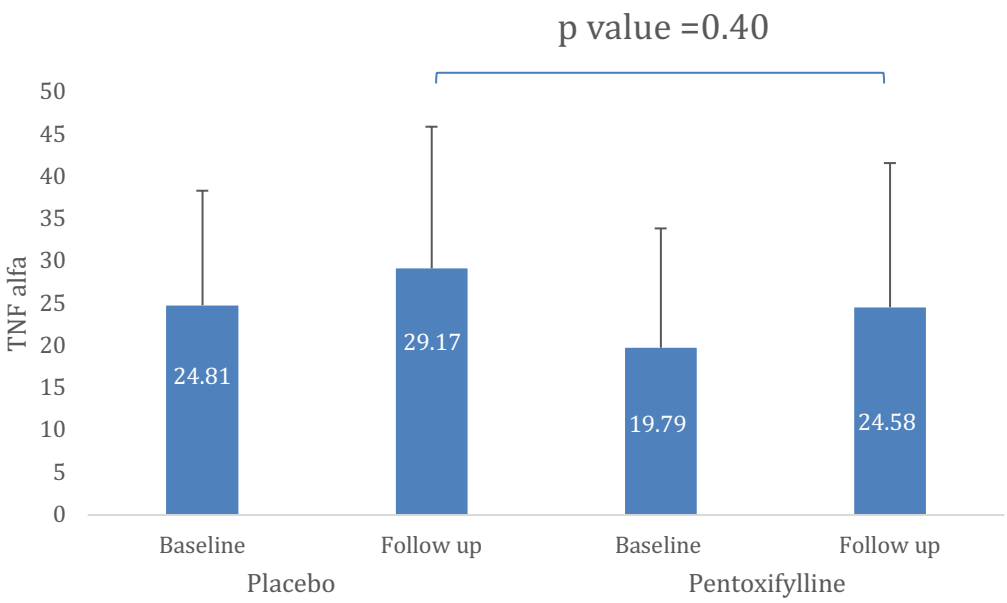


Figure 11.6 b. IL-6 in placebo and pentoxifylline group

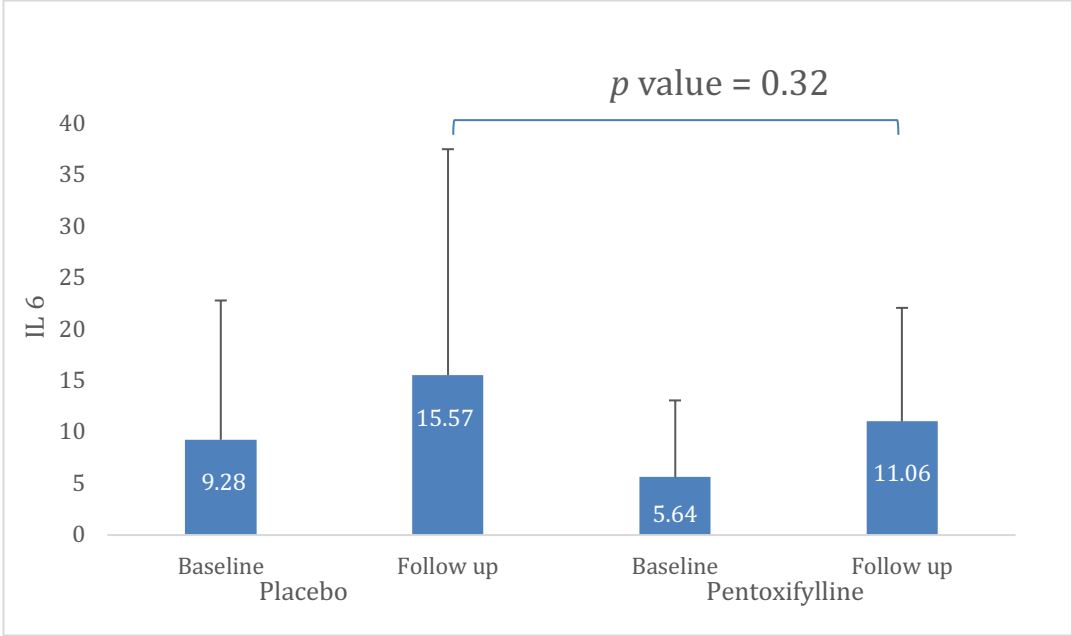


Figure 11.6 c. TGF in placebo and pentoxifylline group

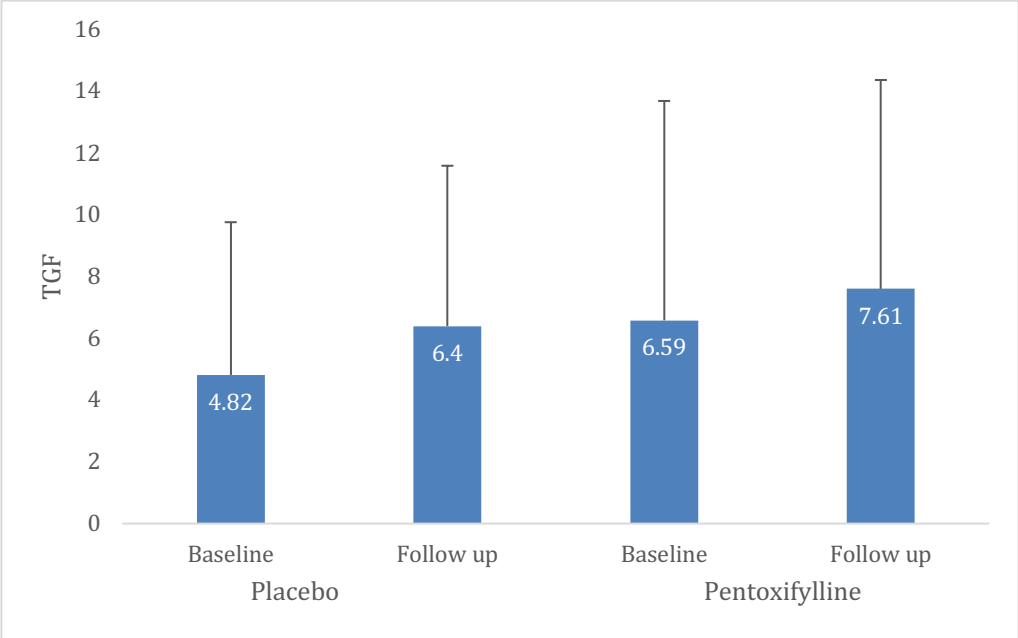
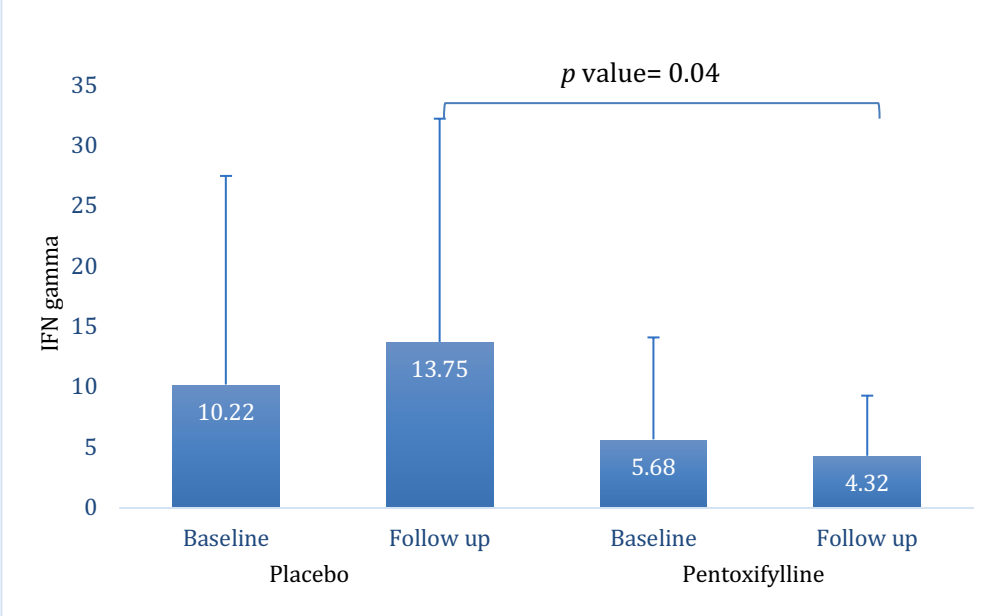


Figure 11.6 d. IFN gamma in placebo and pentoxifylline group



11.5.1 Cytokine variability during the course of study

Figure 11. 7Cytokine variability during the study

Figure 11.7 a & b Interferon gamma (IFN gamma) variability during study in Pentoxifylline and placebo group respectively

Figure 11.7 a

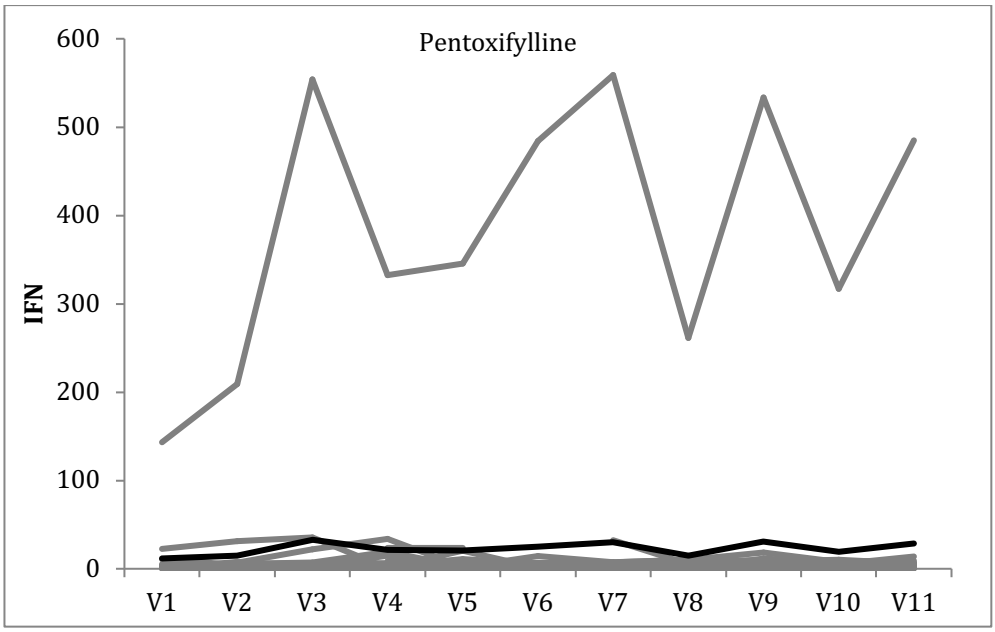
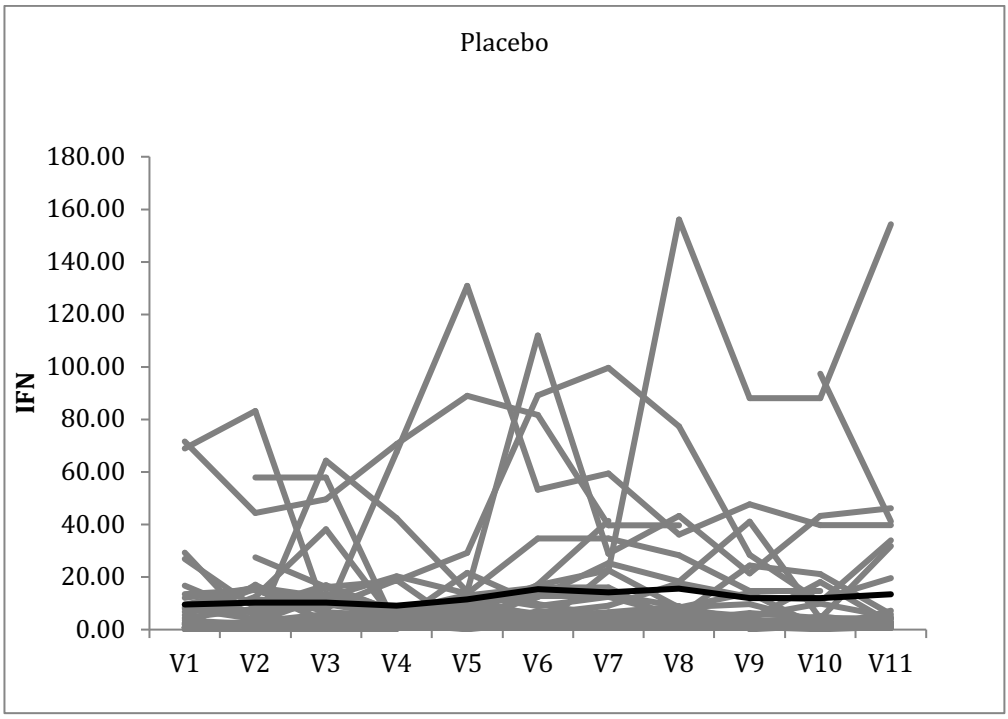


Figure 11.7 b



Figures 11. 7 (a & b) Comparison of IFN gamma variability across Pentoxifylline and Placebo groups. Grey lines show case variability of available results across 11 visits for individual patients while black lines show variability in averaged gradient. The difference in averaged scores similar to the area under the curve represented by dark black lines between two groups was statistically significant (t = 4.58, p<.01) with Pentoxifylline group reporting significantly higher mean averaged score (M = 22.62, SD = 7.28) as compared to Placebo group (M = 12.11, SD = 2.25). Hence, Interferon gamma levels were significantly higher in Pentoxifylline group.

Figure 11.7 c & d Transforming growth factor beta variability during the study in Pentoxifylline and placebo groups respectively

Figure 11.7 c

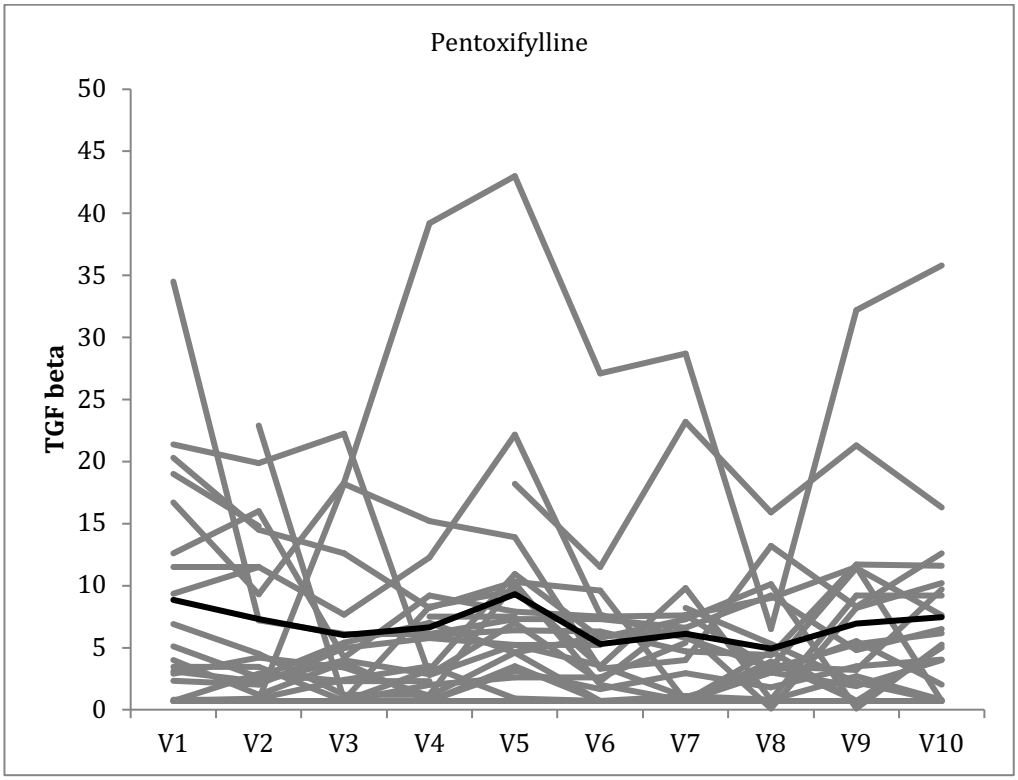
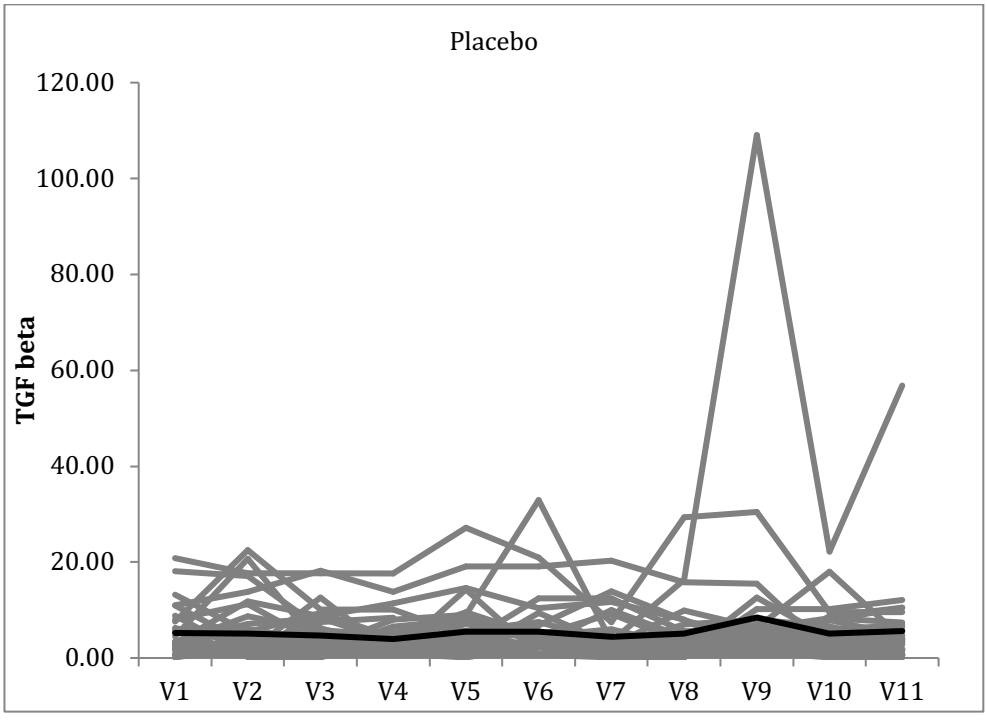


Figure 11.7 d



Figures 11.7 (c & d) Comparison of TGF variability across Pentoxifylline and placebo groups. Grey lines show case variability of available TGF levels across 11 study visits for individual patients while black lines show variability in averaged gradient. The difference in averaged scores similar to the area under the curve, represented by dark black lines between two groups was statistically significant (t = 2.38, p<.05) with Pentoxifylline group reporting significantly higher mean averaged score (M = 6.68, SD = 1.50) as compared to placebo group (M = 5.33, SD = 1.14). Hence TGF beta was significantly higher in pentoxifylline group.

Figure 11.7 e & f IL-6 variability during the study in Pentoxifylline and placebo group respectively

Figure 11.7 e

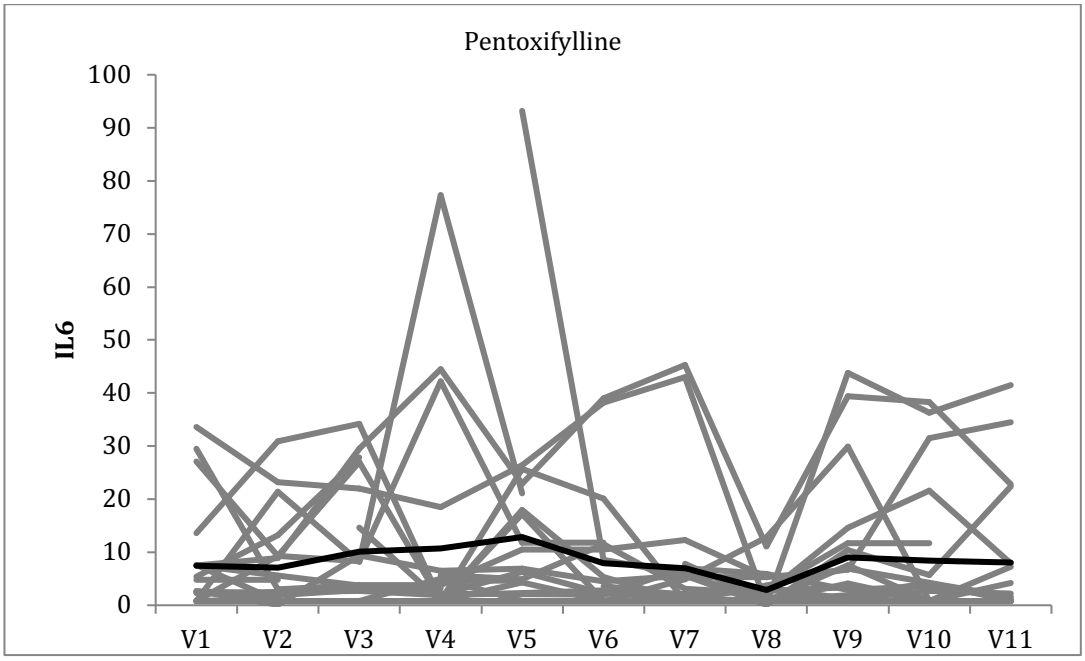
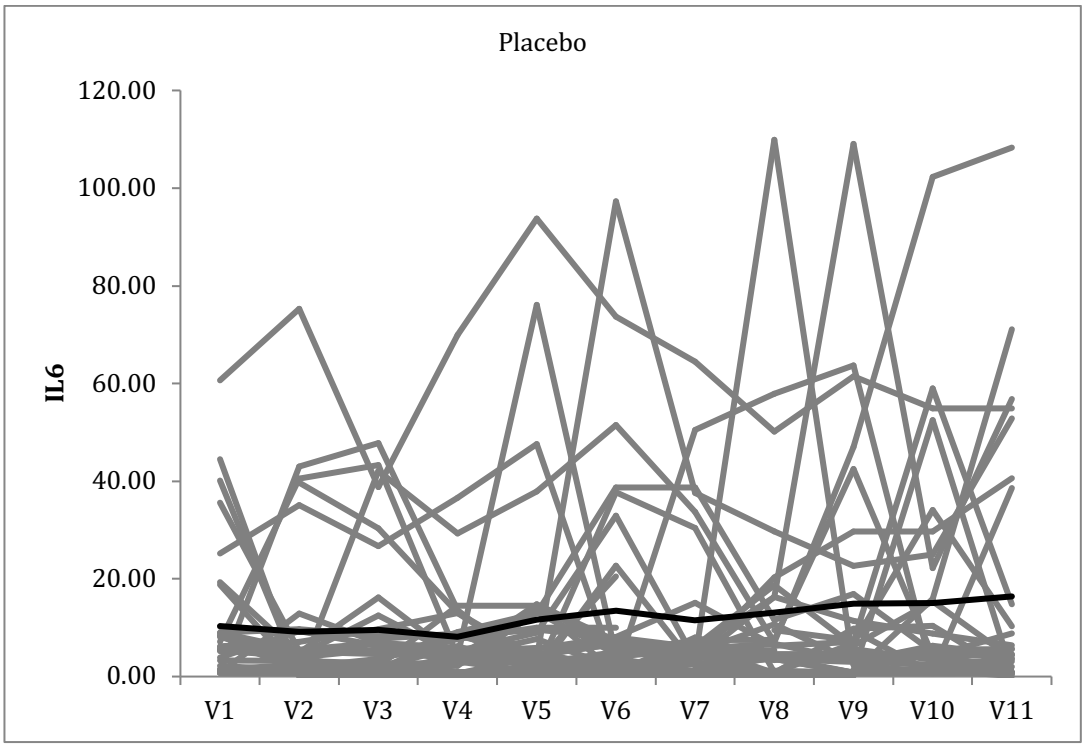


Figure 11. 7 f



Figures 11.7 (e & f)

Comparison of IL-6 variability across Pentoxifylline and Placebo groups. Grey lines show case variability of available IL-6 levels across 11 study visits for individual patients while black lines show variability in averaged gradient. The difference in averaged scores similar to the area under the curve, represented by dark black lines between two groups was statistically significant (t = 3.41, p<.01) with Placebo group reporting significantly higher mean averaged score (M = 12.11, SD = 2.69) as compared to Pentoxifylline group (M = 8.31, SD = 2.52).

Figure 11. 7 g & h TNF alfa variability during the study in Pentoxifylline and placebo groups respectively

Figure 11.7 g

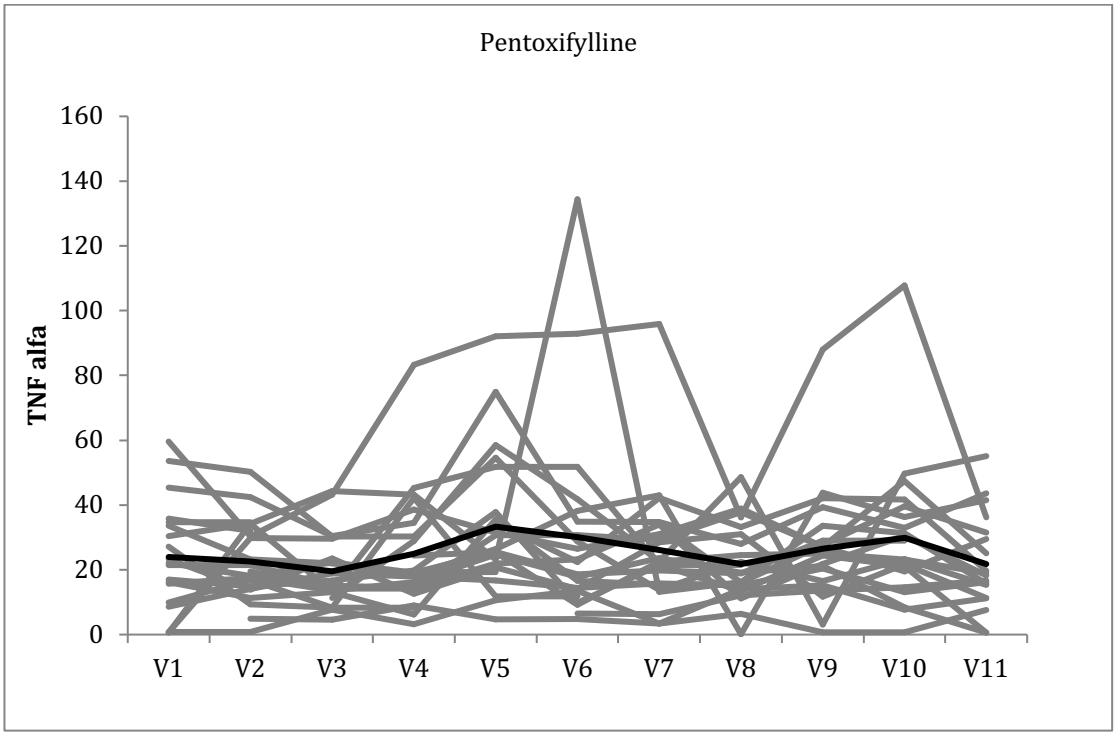
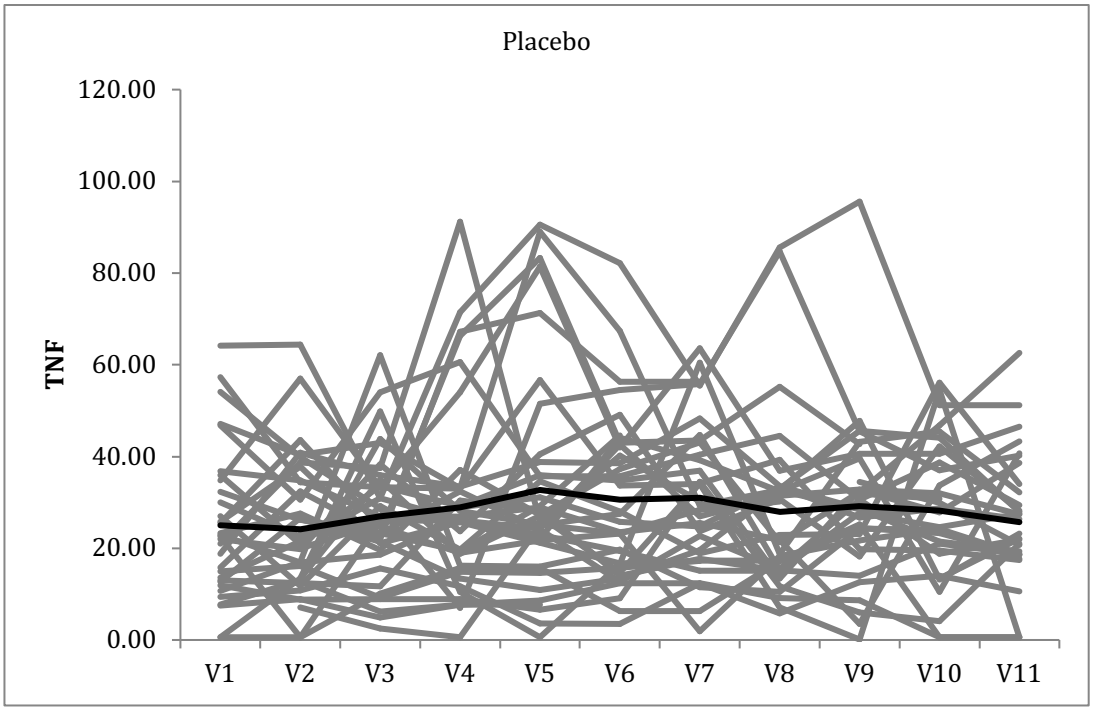


Figure 11.7 h



Figures 11.7 (g & h) Comparison of TNF variability across Pentoxifylline and Placebo groups. Grey lines show case variability of available TNF levels across 11 study visits for individual patients while black lines show variability in averaged gradient. The difference in averaged scores similar to the area under the curve, represented by dark black lines between two groups was statistically not significant (t = 1.77, p = .09) with placebo group reporting mean averaged score (M = 28.19, SD = 2.64) as compared to Pentoxifylline group (M = 25.54, SD = 4.22).

11.6 Safety analysis

Table 11.6 Adverse event description

Adverse event category	**Pentoxifylline group (n=28)**	**Placebo group (n=39)**	***p value***
Others	88	124	<0.05
Cardio vascular system except fluid overload	57	85	<0.05
Fluid overload	29	45	<0.05
Gastrointestinal system	9	22	0.05
Access related - non-infectious	58	63	<0.05
Access - infectious	5	11	0.32
Medication change	40	65	<0.05
Respiratory system	11	23	0.11
Musculoskeletal including – Bone metabolism	9	30	<0.05
Neurological system (including ophthalmology)	13	2	<0.05
Genitourinary system	4	8	0.5
Endocrinology	10	8	0.16
Skin	4	1	0.07
Deaths	2	0	0.09
Serious adverse events	8	16	0.14

Pentoxifylline group had overall less number of adverse events compared to placebo group. Serious adverse events (SAEs) reported in the table are also classified into sub groups except for deaths. None of the serious adverse events were thought to be secondary to IMP during the course of the study.

In Pentoxifylline group, 1 patient underwent blood transfusion as results of complications related to emergency vascular surgery while 3 patients underwent blood transfusions in placebo group. There were 14 incidents of loss of haemodialysis blood circuit among 9 patients in Pentoxifylline group. There were 7 such incidents among 6 patients in placebo group (p value =0.005). Haemodialysis blood circuit clot and blood transfusions were classed under 'Others' adverse event category (Table 10.6).

12. Incidental clinical findings on FDG PET CT scans

Burden of comorbidities is high among haemodialysis patient. Underlying infective focus or occult malignancy is always of concern when patients have Erythropoietin stimulating agent (ESA) hypo responsiveness but otherwise clinically stable. Patients with similar baseline characteristics underwent whole body 15 Fluorodeoxyglucose positron emission tomography along with low dose computerised tomography (FDG PET CT) scan to assess atherosclerotic plaque inflammation at baseline and at six month following treatment with IMP (Pentoxifylline 400 mg OD or Placebo). Any non-research related clinically significant finding was reported to clinical team by research team for further action.

62 patients underwent imaging at baseline. Of these patients 20 (32%) patients had pathological tracer uptake, 8 (12%) patients had suspected malignancy and 2 (0.03%) patients had confirmed diagnosis of malignancy (renal cell carcinoma in the upper pole & moderately active thyroid nodule found to be papillary carcinoma of thyroid).

51 patients subsequently had follow up imaging. Of these, 12 (24%) patients had new evidence of pathological tracer uptake of which 1 (0.02%) patient had new finding suggestive of malignancy requiring interval scan. Only 3 patients still had on going findings noticed on initial imaging. There was no association between elevated non-highly sensitive (hs) c reactive protein (CRP) around time of scan and pathological tracer uptake (P=0.468).

In total 31% scans showed pathological tracer uptake. 7 (6.4%) patients underwent invasive investigations to rule out malignancy. 2 patients had confirmed malignancy, 1 was started on anti -tubercular therapy, 4 patients needed antibiotics and 1 patient underwent colonoscopy for polypectomy. 2 patients who had confirmed malignancy had to be suspended from ongoing living donor renal transplant workup (Table 7). This observation is reflective of disease burden among clinically asymptomatic haemodialysis patients.

Table 12.1. Incidental findings on FDG PET CT scan

Timing of Imaging	Initial	Follow up
Number of scans	62	49
Pathological tracer uptake	20	15
Suspected malignancy	9	1 (new)
Lung nodule	0	1
Inflammatory / infective lymphadenopathy	4	1 (old)
Colonic polyp (non-malignant)	1	
Infected renal cyst	1	
Infected sebaceous cyst	1	
Renal cell carcinoma	1	
Papillary carcinoma of thyroid	1	
Confirmed malignancy (new)	2	2 (old)
Pathological uptake (other)	11	11
Arthropathy	2	2
Sialadenitis	2	1
Access related infection	1	1
Gastric mucosal uptake	2	3
Lung changes	3	2
Lymphadenopathy	0	1
Other	1	1

13. Conclusion and discussion

Anaemia management, particularly in a patient with high ESA requirement poses a challenge to clinicians. High ESA requirement is also associated with adverse cardiovascular outcomes resulting in significant morbidity and mortality in this cohort of patients. Inflammation is one of the major factors contributing to high ESA requirement as well as adverse cardiovascular outcomes.

Pentoxifylline by its effect on inhibition of cytokine production through cAMP pathway may result in the reduction of inflammation. Pentoxifylline has been shown to improve Hb or reduce ESA requirement in a few uncontrolled studies and one double-blind placebo-controlled RCT.

This thesis aimed to assess the effects of Pentoxifylline on ESA resistance and associated outcomes. We also explored the effect of pentoxifylline on surrogate markers of adverse cardiovascular events such as aortic distensibility and vascular plaque inflammation measured by cardiac MRI and FDG PET CT scan respectively. Effect of pentoxifylline on inflammatory status was also assessed.

13.1 Effect of Pentoxifylline on ESA requirement

This double-blind placebo controlled randomised study did not show any statistically significant beneficial effect of pentoxifylline compared to placebo in reducing ESA requirement (reflected by ESA/Hb ratio, ESA dose, Hb levels and ERI) in ESA resistant haemodialysis patients. Baseline characteristics were similar in both groups except for higher haemoglobin levels in Pentoxifylline group (p value = 0.01) and corrected calcium levels (p value = 0.02) in the placebo group. The impact of higher haemoglobin level may have been significant if the study results were positive**.** The Patient cohort was ethnically diverse, and a majority of patients were male, reflecting patient distribution at our centre.

There was an improvement in the primary outcome measure of ESA / Hb ratio in the Pentoxifylline group compared to the placebo group, but it failed to reach statistical significance. A similar trend was noted within the Pentoxifylline group when baseline ESA/Hb ratio was compared with follow up results.

One patient underwent blood transfusion in pentoxifylline group while three patients received blood transfusion in the placebo group. There was a significantly higher incidence of haemodialysis blood circuit loss due to clotting in the Pentoxifylline group compared to the placebo group (p value =0.005). Dialysis circuit was discarded by the clinical team for one patient each in both groups to prevent haemodialysis access from getting thrombosed due to Hb greater than 13gm/dl (off protocol). Each episode of dialysis circuit loss results in 200-300 ml of blood loss leading to drop in Hb levels. This factor could have also contributed to the lack of statistically significant outcomes.

Despite a robust study design, this study was limited by less than expected recruitment of patients. Investigators felt that there were many barriers to recruitment in a trial with an investigational medicinal product. Already high pill burden, known poor compliance to treatment as a result of various psychosocial aspects of chronic illness were some of the main reasons behind the lack of acceptance among patients. Cultural and language barriers also play a role in relatively poor research study acceptance among our cohort.

After the completion of the current study, there has been a drive by various agencies at the national and local level to enhance research awareness among CKD patients. Higher patient recruitment rates to other trials were noted after these interventions.

The results of our study are in contrast to another double-blind placebo controlled RCT published after the commencement of our study. In this multicentre trial, 53 patients with CKD 4 or 5D with ESA hyporesponsiveness defined by ESA resistance index of ≥10.0 I.U./Kilograms (kg)/wk/gm/dL for erythropoietin-treated patients and ≥ 0.05 mg/kg/wk/gm/dL (for darbepoetin

alpha-treated patients) were given pentoxifylline 400mg once a day (OD) for four months. There was no significant improvement in ERI between the pentoxifylline and control group (p=0.1). However, pentoxifylline significantly improved Hb concentration relative to the control group (p=0.01). No difference in adverse events was noted in either group. This trial was limited by a smaller sample size than planned due to slow recruitment. This study also included patients with CKD stage 4 [123].

A randomised study from Isfahan, Iran, Mortazavi et al. compared the effect of pentoxifylline on haemoglobin and ESA required compared to placebo. 50 patients undergoing chronic haemodialysis enrolled in the study were divided into two groups taking pentoxifylline and placebo over a period of six months. The Study did not show any significant change in haemoglobin (10.6+1.4 vs 10.1+1.7) or ESA requirement (7.5+4.4 vs 8.3+3.4) between pentoxifylline and placebo group respectively. Unfortunately, this study publication was marred by lack of details on study methodology and baseline characteristics. The baseline characteristics were only compared for age, sex and cause of renal failure. The study did not enrol patients with ESA resistance. Study methodology such as randomisation or any blinding protocol was also not mentioned. There is a possibility that there were many confounding factors that could have resulted in the published negative results. Therefore drawing any valuable conclusion from this study may be inappropriate [126].

In a non-blinded study published by Shahbazian et al demonstrated a statistically significant improvement in haemoglobin in ESA resistant hemodialysis patients treated with pentoxifylline compared to controls. Hb was reported to be 11.22 +/- 1.26 in treatment group (n=19) compared to 9.77 +/- 1.08 in control group (n=20) with a p value of 0. 001.Within the groups there was a statically significant improvement in CRP in treatment group. Unfortunately, ESA dosage comparison between the groups at baseline or at the end of treatment was not published. Although results of this study were in line of available data on pentoxifylline yet it will be difficult to interpret the results in the absence of ESA dosage[127].

On the other hand, smaller uncontrolled studies discussed in the introduction section, have demonstrated improvement of ESA requirement and or haemoglobin. Studies including a double-blind placebo-controlled studies have demonstrated pentoxifylline associated reduction in inflammation status in CKD patients by reduction in cytokines and highly sensitive CRP levels[8, 112, 113]. This is in keeping with the hypothesis of inflammation-driven ESA hyporesponsiveness through direct inhibition of erythropoiesis and functional iron deficiency through the hepcidin pathway.

Randomised controlled studies are not able to demonstrate the effectiveness of pentoxifylline in ESA resistance. This could be attributed to comorbidities associated with advanced CKD patients on haemodialysis who subsequently suffer from ESA resistance. This high-risk cohort of patients suffers from frequent spells of illnesses usually associated with cardiovascular events and infective complications which could mask the potential beneficial effect of Pentoxifylline resulting from its effect on pro-inflammatory cytokines demonstrated in our study. Pentoxifylline was consistently found to be safe in haemodialysis patients.

Recently, the effect of pentoxifylline was studied in another inflammatory condition. A large multicentre, double blind study involving 1103 patients evaluated the effect of pentoxifylline on acute alcoholic hepatitis compared to prednisolone. The dose of Pentoxifylline used was also 400 mg OD. This study did not show any beneficial effect of pentoxifylline compared to Prednisolone. Prednisolone was shown to improve short-term outcome in acute alcoholic hepatitis [128].

There is growing evidence that Diabetic nephropathy is an inflammatory condition. Therefore, newer anti-inflammatory agents have been studied to halt the progression of the disease. The PREDIAN trial studied the impact of anti-inflammatory and anti-fibrotic effect of Pentoxifylline in patients with Diabetic nephropathy who were on renin-angiotensin system (RAS) inhibition therapy. In this open label randomised study, 82 patients received Pentoxifylline 1200 mg

once a day while 87 patients were assigned to the placebo group for a period of 2 years. There was a statistically significant lesser reduction of GFR in the Pentoxifylline group compared to placebo at the end of the two year study period. Urinary TNF alfa also improved significantly in Pentoxifylline group. The authors mention that improving trend of GFR was apparent at six months and reached a level of statistical significance only after one year of therapy. Pentoxifylline at the given dose was tolerated well except for higher incidence of gastrointestinal symptoms which resolved spontaneously in the majority of patients. Hence, a higher dose of Pentoxifylline given for longer duration has shown significant benefits in diabetic nephropathy including a reduction in urinary cytokines levels[129].

On face value PEAR study may appear negative albeit dose used was 400 mg and duration of the study was six months which may have been too short to show any improvement in ESA requirement despite some beneficial effects on overall cytokine exposure. The effect of Pentoxifylline was not translated into its beneficial effect on ESA requirement. Improving trends were also visible in atherosclerotic plaque inflammation quantified by FDG PET CT analysis. There is a possibility that a longer duration of study and or higher dose of Pentoxifylline, could have shown a beneficial effect on the primary outcome. Unfortunately, the quality of life assessment was not done during this study.

PEAR study outcomes make a strong argument for a large outcome based study conducted in a setting of a pragmatic, large cluster randomised trial. Higher dosing of pentoxifylline could be considered in haemodialysis patients following an appropriate pilot study. Chronic haemodialysis setting is an ideal situation for conducting pragmatic clinical trials. While undergoing haemodialysis, patient encounters are regular and predictable. A significant amount of clinical data is already collected for routine clinical care. National renal registries also collect various aspects of this dataset in the majority of the developed world [130].

In conclusion, further studies are required with larger patient numbers to establish effect pentoxifylline for ESA hyporesponsiveness in haemodialysis

patients. Two- well designed RCTs have failed to recruit adequate numbers. There is a scope to consider to large pragmatic trial with randomisation by 'cluster' rather than individual patient-based randomisation.

13.2 Effect of pentoxifylline on surrogate markers of cardiovascular risks and inflammation status

The incidence of Chronic kidney disease is on the rise across the world. In 2010, CKD was the third biggest cause of overall loss of years of life (82%) behind HIV & AIDS (396%) and diabetes mellitus (93%) [131]. Studies have shown the association of a reduction in GFR with the rise in cardiovascular disease risk. This risk is even higher in patients undergoing haemodialysis [132]. In addition to traditional risk factors, an inflammatory state in CKD is associated with adverse cardiovascular outcomes.

13.2.1 Atherosclerotic plaque inflammation

The impact of arterial wall inflammation on increased cardiovascular disease risk in CKD patients is coming into the spotlight over the past few years. The focus of intervention studies has shifted to metabolically active inflamed unstable plaque rather than calcified, stable but anatomically significant atherosclerotic plaque.

Interventions such as the statins, which cause the reduction in atherosclerotic plaque inflammation have resulted in improvement in cardiovascular outcomes in non-dialysis patients. The method of analysing the anti-inflammatory effect of an intervention on atherosclerotic plaque inflammation with 18 FDG PET CT scan has been well established with statin trials.

A multicentre randomised placebo-controlled double blind study by Tawakol et al. demonstrated the effect of intensifying statin therapy on atherosclerotic plaque inflammation along the major blood vessels. 83 patients with cardiovascular disease risk factors or known to have atherosclerosis, were randomised to atorvastatin 10 mg versus 80 mg OD dosing. FDG PET CT imaging analysed atherosclerotic plaque inflammation in carotid arteries and ascending aorta by measuring TBR of FDG uptake in the plaques at baseline, four weeks and twelve weeks interval. Statins produced a dose-dependent significant reduction in plaque inflammation noted as early as four weeks scan which continued to

improve at 12 weeks scans. There was no association of highly sensitive CRP or lipid profile with these changes [97].

In haemodialysis patients, statins have not been found to be effective in reducing cardiovascular events in multiple well designed trials[133-136]. This is probably due to the complex mechanism and a higher degree of inflammation in haemodialysis patients.

Due to its anti-inflammatory action in dialysis patients, the effect of pentoxifylline on atherosclerotic plaque inflammation was also studied. PEAR study was the first study to quantify atherosclerotic plaque inflammation in chronic haemodialysis patients. The analysis of baseline scans (before intervention) of all patients showed a significantly elevated arterial wall FDG uptake with a mean (SD) TBR value of 5.9 (2.93). To put this observation of high mean TBR into perspective, as also mentioned in the introduction section, a study of non-CKD patients undergoing FDG PET CT scan, mean (SD) TBR value of 2.27 (0.34) was associated with risk of developing cardiovascular disease in 6 months [95].

The improvement of atherosclerotic plaque inflammation depicted by TBR in the Pentoxifylline group failed to reach statistical significance compared to the placebo group after six months of treatment. There was no statistically significant difference between the two groups for SUV max as well. In the Pentoxifylline group, there was an improvement of TBR at the time of follow up scan when compared with baseline scans. While in the placebo group, there was deterioration of TBR in follow up scans when compared to baseline scans. Therefore, even though statistically nonsignificant, the positive impact of Pentoxifylline demonstrated by PEAR study, in reducing or halting atherosclerotic plaque inflammation in haemodialysis patients could still be inferred.

13.2.2 Cardiac MRI

This study did not show any statistically significant change in Aortic compliance between pentoxifylline and placebo group. There were no significant changes in parameters of cardiac function such as end-diastolic volume, end systolic volume, stroke volume, ejection fraction, myocardial mass when compared between two groups at the end of 6-month treatment period.

The cardiac MRI analysis was limited by a very small number of patients undergoing imaging. Major barriers to patient participation were a) new diagnosis of claustrophobia while undergoing scans b) patients unable to fit into cardiac MRI scanner as a result of obesity. Some patients did not attend imaging due to lack of available time. Haemodialysis patients undergo dialysis treatment three times per week and remaining two working days of the week are usually taken up with tasks such as GP visits, other hospital appointments or personal work.

In haemodialysis patients, increased aortic stiffness is a strong independent risk factor for cardiovascular and all-cause mortality [108, 109]. Study by Briet et al. demonstrated that GFR was strongly correlated with arterial stiffness [136]. Another study demonstrated that 24 patients on chronic haemodialysis with no history of coronary heart disease had significantly reduced aortic compliance similar to non-CKD patients with advanced coronary artery disease [137].

As previously discussed in the introduction section, arterial stiffness is the outcome of a complex process of alteration in the intrinsic elastic properties as result of calcification, increased collagen content, elastinolysis, reduced density of vascular smooth muscle cells and inflammation. In ESRD, calcification of blood vessels plays a significant role in the pathogenesis of arterial sclerosis. Hence the interventions which are proven to be effective in improving arterial stiffness in non-CKD population may not be beneficial in advanced CKD patient cohort. Atherosclerosis is not considered to be the major contributor towards arterial stiffness.

Blood pressure has been considered as a modifiable risk factor in the pathogenesis of arterial stiffness, but its role in ESRD patients remains uncertain. A study of 150 patients with ESRD demonstrated that pulse wave velocity which was not sensitive to changes in BP control was an independent predictor of mortality. In this study, BP control was optimised by adjusting dry weight and pharmacological treatment such as ACE inhibitor, calcium antagonists and beta blockers. Patients were followed up for mean 58 months. Lack of reduction in pulse wave velocity in response to an improvement in BP was associated with cardiovascular and all-cause mortality. Adjusted RR for a PWV decrease of 1 m/s was 0.79 (95% CI 0.69 to 0.93) for cardiovascular mortality and 0.71 (95% CI 0.60 to 0.86) for all-cause mortality. Use of an ACE inhibitor had a favourable response on all cause and cardiovascular mortality [138].

A recent study published by Sarafidis et al analysed the significance of ambulatory BP, central pulse pressure, pulse wave velocity and heart rate adjusted augmented index in 170 haemodialysis patients. At the end of average follow up of 28 months, ambulatory pulse wave velocity was the only parameter associated with the primary end point of all-cause mortality and non-fatal cardiovascular events (hazard ratios, 1.579; 95% confidence intervals, 1.187-2.102) [139].

Inflammation plays a significant role in the pathogenesis of arterial stiffness. Inflammation measured by hs CRP and cytokine levels have been shown to be associated with arterial stiffness in non-CKD patients. [140, 141]. In inflammatory conditions secondary to rheumatological problems, interventions targeted towards reducing inflammation status have resulted in improvement of arterial wall stiffness [105].

Similar to hepcidin in iron regulation, the role of Fetuin in arterial stiffness is worth mentioning. Fetuin is a negative acute phase protein synthesised in the liver. It inhibits calcification of blood vessels by maintaining solubility of serum calcium phosphate. Fetuin leads to formation of calciprotein particles which in turn result in inhibition of calcium phosphate aggregate formation [142].

A study of 222 prevalent haemodialysis patients by Metry et al , demonstrated increased mortality risk (HR 2.3; CI 1.2-4.5, P = 0.01) in inflamed haemodialysis patients in with low Fetuin-A protein levels compared to non-inflamed dialysis patients with high Fetuin-A protein levels after adjustment for comorbidities score, age, gender, dialysis vintage and inflammation [143]. Multiple comorbidities such as diabetes mellitus, hypertension and pre-existing inflammatory illnesses in the presence of conditions associated with haemodialysis such as inflammation, oxidative stress and deranged bone mineral metabolism provide a perfect stage for the pathogenesis of arterial stiffness.

In PEAR study, we were limited by the number of patients undergoing the gold standard test for measurement of Pulse wave analysis. Therefore, we were we were unable to establish any trends for changes in various cardiovascular parameters. However, there is scope for further studies focussed on the impact of reducing inflammation load in dialysis patients on arterial stiffness by conducting longer duration of studies as alluded to earlier. Interventions to reduce inflammation in advanced CKD remains a relatively unexplored area in CKD research.

13.2 Effect of pentoxifylline on cytokines

Treatment of ESA resistant haemodialysis patients with pentoxifylline did not show any statistically significant reduction in cytokine titres, compared to placebo when analysed based on primary outcome analysis criteria. There was an improving trend of IFN gamma in the Pentoxifylline group when values at the start and the end of study were compared.

Our cohort of patients had a very high incidence of infective or inflammatory origin adverse events. These adverse events would in-turn affect the cytokine titres leading to high variability in results. Therefore comparing overall trends with averaged scores would provide a better picture of cytokine levels during

the course of the study. On comparing the variability during the overall study period, IL-6 and TNF alfa titres showed an improvement in the Pentoxifylline group. The difference of averaged score of IL -6 levels achieved statistical significance when compared to placebo was suggestive of reduced inflammatory burden in the Pentoxifylline group. The anti-inflammatory cytokines, TGF beta and IFN gamma titres, on the other hand, were higher in pentoxifylline compared to placebo group when comparing the overall variability between the groups. These results are in line with general anti-inflammatory properties of Pentoxifylline.

In PEAR study, Pentoxifylline has been shown to reduced exposure of IL-6 and TNF levels for a period of six months. While exposure of anti-inflammatory cytokines such as TGF beta and interferon gamma was higher in Pentoxifylline during the study period. These results suggest an overall reduction in the inflammatory milieu in Pentoxifylline patients.

An excellent meta-analysis by Brie et al. reviewed the effect of pentoxifylline on inflammatory markers and blood pressure. 15 randomised controlled trial studies with 16 treatment arms were included in the analysis. Only one study included patients with chronic kidney disease [144] while the rest of the studies included patients with known cardiovascular disease and associated risk factors such as diabetes mellitus and associated proteinuria. There was conclusive evidence on the effect of pentoxifylline in reducing TNF alfa (Weighted mean difference (WMD): -1.03 pg/ml, 95% CI: -1.54, -0.51; $P<0.001$ in 11 treatment arms) and hs CRP (WMD: -1.39mg/l, 95% CI: -2.68, -0.10; $P=0.034$ in 5 treatment arms). The meta-analysis could not demonstrate any significant reduction in IL- 6 titres or blood pressure.

However, the role of pentoxifylline on inflammatory status measured by cytokines in advanced CKD patients has not been consistent. Two small non randomised studies by Cooper et al. (3) and Ferrari et al. (104) demonstrated a reduction in TNF-α, interferon gamma and IL-6 respectively. Another uncontrolled study involving 15 ESA resistant stable haemodialysis patients

showed pentoxifylline associated improvement in HB levels with no significant impact on TNF-α titres over a period of 3 months [145]. As previously discussed, double-blind RCT by Gonzalez-Espinoza et al. demonstrated a statistically significant reduction in TNF-α, IL-6 and C reactive protein 18 haemodialysis patients with the matched controls. Unfortunately, the outcome did not include Hb concentration or ESA dose follow up. Reduction of specific inflammatory markers may not necessarily correlate with improvement of overall multifactorial inflammation unless there is an improvement in associated outcomes such as improvement in ESA resistance or cardiovascular risk (or surrogate markers).

The PEAR study is the only double-blind placebo controlled study to investigate the effect of Pentoxifylline on ESA resistance, surrogate markers of cardiovascular risks and inflammation status. Once again similar to PET CT imaging studies the improving trend of pro and anti-inflammatory cytokines is promising.

13.3 Effect of pentoxifylline on CKD progression and proteinuria: a review of available evidence

The effect of pentoxifylline on the progression of CKD and proteinuria has investigated in multiple studies over past 2 decades. The beneficial effect of pentoxifylline on CKD is through its anti-inflammatory activity described earlier in the thesis. Pentoxifylline has been shown to reduce urinary TNF alfa in proteinuric diabetic kidney disease [146] and suppresses Monocyte chemoattractant protein-1 in non-diabetic chronic kidney disease [147]. In in-vitro studies by Lin et al, Pentoxifylline has also been shown to attenuate renal progression in rats with remnant kidneys [148]and reduce renal fibrosis by blocking SMAD 3/4 activated transcription of connective tissues growth factor [149].

In a post hoc analysis of PREDIAN trial pentoxifylline was shown to be associated with decreased serum and urinary TNF alfa while there was a significant increase in serum and urinary Klotho after 1 year of therapy. Kidney

is the main source of klotho and its level is significantly decreased in CKD. Klotho by its actions as a humoral factor regulates various signalling pathways with beneficial effects towards reducing progression of CKD[150].

Metanalysis published by Jiang et al showed that Pentoxifylline significantly decreased proteinuria [weighted mean difference (WMD) -0.60 g/day (95 % CI -0.84 to -0.36); $p < 0.001$] compared to placebo or no-treatment groups, but the reduction in proteinuria was not statistically significant when compared to ACE inhibitor (captopril) treatment. The decrease of glomerular filtration rate was significantly less [WMD: 3.67 ml/min (2.71-4.62); $p < 0.001$] in the pentoxifylline group than in the controls. This study included 12 trials with 613 participants. Inclusion criteria was prospective randomised and non-randomised control trials including patients with CKD and proteinuria. Minimum follow up duration was 2 months. This metanalysis was limited by studies with high degree of heterogeneity with respect to dose and duration of pentoxifylline administration, staging and cause of CKD, degree of proteinuria, usage of ACEI and ARBs. Cause of CKD in majority of the studies was diabetic nephropathy. Majority of studies were classed as low methodological quality and short follow up duration[151].

In a metanalysis published in 2016 by Leporini et al, pentoxifylline was shown to improve renal function, particularly in advanced stages of CKD in studies with long follow up period. The improvement in proteinuria was more evident in patients with type 1 diabetes and with high degree of proteinuria. This metanalysis included 26 studies with 1518 participants. All the studies analysed by Jiang et al were also included in this metanalysis. The inclusion criteria were RCTs and quasi RCTs providing information on the effects of Pentoxifylline on renal endpoints in CKD patients. The follow up period in the studies analysed, ranged from 21 days to 12 months except for the PREDIAN study which had follow up period of 24 months. Rest of the limitations encountered were similar to limitations described by Jiang et al. Pentoxifylline was shown to be well tolerated in patients with CKD [152].

RAAS inhibition with ACE inhibitor or an ARB has been proven as gold standard treatment for delaying the progression of both diabetic and non-diabetic CKD for many years[153-155]. A large prospective cohort study from a national insurance registry-based data has shown beneficial effect of pentoxifylline in reducing risk for composite outcome of maintenance dialysis initiation or death in patients already on ACE inhibitor (HR, 0.94; 95% CI 0.90-0.99) or ARB treatment (HR, 0.91; 95% CI, 0.85-0.97), after propensity score matching. The study enrolled 14,117 with CKD stage 5 (not on dialysis) with creatinine >6 mg/dl and hematocrit <28% and on pentoxifylline treatment[156]. Another similar national insurance cohort study published by Wu et al demonstrated that pentoxifylline provided comparable beneficial effect when combined with ACE inhibitor or ARB therapy. Pentoxifylline was also shown provide greater benefit in slowing down progression of CKD in patients with chronic kidney disease stage 5 who were not on dialysis[157].

A metanalysis published in 2017 on the effect of pentoxifylline therapy combined with ACE inhibitor or ARB on CKD progression and proteinuria demonstrated protective effect of pentoxifylline in patients with CKD stage (3-5) and proteinuria when compared to ACE inhibitor or ARB therapy alone. Combined therapy showed significant improvement in proteinuria (SMD -0.52; 95% CI -0.90 to 0.15; $I^2 = 68\%$) and significant improvement in rate of CKD progression (SMD 0.30; confidence limit [Cl] 95% CI 0.06 to 0.54; $I^2 = 0\%$) at 6 months of therapy. The beneficial effect was also noted at 9 to 12 months. This metanalysis included 11 randomised controlled studies with 705 enrolled patients[158].

The role of pentoxifylline is promising in CKD progression. However, none of the studies reported hard outcomes such doubling of serum creatinine, need for renal replacement therapy, cardiovascular outcomes and mortality as primary outcome measure. Therefore, well planned multi centre trials are needed before pentoxifylline could be recommended to be used in CKD patients.

13.4 Safety analysis

In keeping with available data on pentoxifylline usage in haemodialysis patients, drug was noted to be safe. None of the adverse events in the pentoxifylline group were attributed to pentoxifylline. As predicted in this patient cohort, most of the hospital admissions leading to SAEs were related to cardiovascular events.

One patient each in both groups had an episode of gastrointestinal bleeding. The patient in the pentoxifylline group was thought to have Mallory Weiss syndrome while patient in the placebo group had not taken any dose IMP voluntarily prior to episode of GI bleed.

There was a significantly higher incidence of haemodialysis blood circuit clotting leading to approximately 180 to 300 ml blood per episode. Haemodialysis blood circuit clotting is a multifactorial phenomenon. Hence, pentoxifylline therapy cannot be directly implicated until there is further evidence. This adverse event has not been reported in prior studies.

There was a statistically significant incidence of adverse events under a broad subgroup of neurological system (including ophthalmology) in the pentoxifylline group. Out of a total 15 reported adverse events, 5 adverse events were related to a patient undergoing glaucoma surgery, 2 adverse events were due post dialysis headache, 1 due to presumed transient ischaemic event, 2 due to delirium secondary to sepsis, 2 due to a patient diagnosed with carpal tunnel syndrome, 1 patient required psychological support, 1 patient suffered from cerebrovascular accident (CVA) and one patient was diagnosed with length dependent axonal sensory motor polyneuropathy.

The haemodialysis patient cohort with ESA hyporesponsiveness has a significantly higher comorbidities leading frequent change in medications. The majority changes in medications among study patients included phosphate binders, activated vitamin D, anti-anginals and antibiotics. The higher degree of adverse events in the control group Paragraph 4would explain greater number of medication changes in this group. Relatively less number of adverse event in

pentoxifylline group could be explained by its anti-inflammatory and hemorheological properties.

There were two deaths in the pentoxifylline group. One patient had sudden death at home post dialysis session. Cause for death was presumed to be sudden cardiac death which is unfortunately the commonest cause of sudden death among haemodialysis patients [159]. While the second death was due to lower respiratory tract infection as a complication of CVA.

13.5 Conclusion

Inflammation is increasingly implicated to be the common pathway of most of the cardiovascular risk factors. Pentoxifylline through its properties of improving blood rheology and anti-inflammatory activity has been studied in a variety of conditions associated with inflammation and blood circulation. A review article by McCarty et al. summarises the ongoing research and benefits of Pentoxifylline on a variety of conditions such as prevention of cardiovascular events including control of stable angina, prevention of stroke and transient ischaemic attacks. Similarly there is growing evidence of beneficial effects of Pentoxifylline on the progression of chronic kidney. Perioperative administration of Pentoxifylline administration has also been shown decrease systemic inflammatory response after cardiopulmonary bypass surgery as demonstrated by reduced neutrophil activation and pro-inflammatory cytokine response. Unfortunately, most of the trials on Pentoxifylline are relatively small and have not contributed to any significant change in clinical practice. Pentoxifylline, being a safe, cheap and widely available non-specific anti-inflammatory agent is likely to continue as a compound of immense interest for future research [160].

In the current scenario, there is no proven specific intervention available to improve Cardiovascular outcomes in haemodialysis patients. The available evidence which is driven from small, short-term studies has not led to any significant change in practice in the CKD population. Therefore, targeting arterial wall inflammation in long-term event driven studies may result in substantial

evidence and greater generalizability of findings.

Although outcomes are statistically insignificant yet the data from PEAR study provides an insight into the severity of incidental atherosclerotic plaque inflammation and impact of non-specific anti-inflammatory drug intervention in high-risk haemodialysis patients for the first time.

Bibliography

1. Semenza, G.L., *Oxygen sensing, homeostasis, and disease.* N Engl J Med, 2011. **365**(6): p. 537-47.
2. Azizi, M.H., T. Nayernouri, and F. Azizi, *A brief history of the discovery of the circulation of blood in the human body.* Arch Iran Med, 2008. **11**(3): p. 345-50.
3. Patwardhan, K., *The history of the discovery of blood circulation: unrecognized contributions of Ayurveda masters.* Adv Physiol Educ, 2012. **36**(2): p. 77-82.
4. Ranhel, A.S. and E.T. Mesquita, *The Middle Ages Contributions to Cardiovascular Medicine.* Braz J Cardiovasc Surg, 2016. **31**(2): p. 163-70.
5. Friedland, G., *Discovery of the function of the heart and circulation of blood.* Cardiovasc J Afr, 2009. **20**(3): p. 160.
6. Martins e Silva, J., *From the discovery of the circulation of the blood to the first steps in hemorheology: part 2.* Rev Port Cardiol, 2009. **28**(12): p. 1405-39.
7. Schwarz, H.P. and F. Dorner, *Karl Landsteiner and his major contributions to haematology.* Br J Haematol, 2003. **121**(4): p. 556-65.
8. Cooper, A., et al., *Pentoxifylline improves hemoglobin levels in patients with erythropoietin-resistant anemia in renal failure.* J Am Soc Nephrol, 2004. **15**(7): p. 1877-82.
9. Diez-Silva, M., et al., *Shape and Biomechanical Characteristics of Human Red Blood Cells in Health and Disease.* MRS Bull, 2010. **35**(5): p. 382-388.
10. Jensen, F.B., *The dual roles of red blood cells in tissue oxygen delivery: oxygen carriers and regulators of local blood flow.* J Exp Biol, 2009. **212**(Pt 21): p. 3387-93.
11. Hoffman, R., *Hematology basic principles and practice*. 2013, Saunders/Elsevier,: Philadelphia, PA. p. 1 online resource (xxxi, 2343 p.).
12. D'Andrea, A.D., H.F. Lodish, and G.G. Wong, *Expression cloning of the murine erythropoietin receptor.* Cell, 1989. **57**(2): p. 277-85.
13. Suzuki, N., et al., *Erythropoietin production in neuroepithelial and neural crest cells during primitive erythropoiesis.* Nat Commun, 2013. **4**: p. 2902.
14. Pan, X., et al., *Isolation and characterization of renal erythropoietin-producing cells from genetically produced anemia mice.* PLoS One, 2011. **6**(10): p. e25839.
15. Wu, H., et al., *Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor.* Cell, 1995. **83**(1): p. 59-67.
16. Fairbairn, L.J., et al., *Suppression of apoptosis allows differentiation and development of a multipotent hemopoietic cell line in the absence of added growth factors.* Cell, 1993. **74**(5): p. 823-32.
17. Koury, M.J. and M.C. Bondurant, *Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells.* Science, 1990. **248**(4953): p. 378-81.

18. Sui, X., et al., *Erythropoietin-independent erythrocyte production: signals through gp130 and c-kit dramatically promote erythropoiesis from human CD34+ cells.* J Exp Med, 1996. **183**(3): p. 837-45.
19. Malik, J., et al., *Erythropoietin critically regulates the terminal maturation of murine and human primitive erythroblasts.* Haematologica, 2013. **98**(11): p. 1778-87.
20. Wang, G.L., et al., *Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension.* Proc Natl Acad Sci U S A, 1995. **92**(12): p. 5510-4.
21. Manalo, D.J., et al., *Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1.* Blood, 2005. **105**(2): p. 659-69.
22. Wang, G.L. and G.L. Semenza, *Purification and characterization of hypoxia-inducible factor 1.* J Biol Chem, 1995. **270**(3): p. 1230-7.
23. Galson, D.L., et al., *The orphan receptor hepatic nuclear factor 4 functions as a transcriptional activator for tissue-specific and hypoxia-specific erythropoietin gene expression and is antagonized by EAR3/COUP-TF1.* Mol Cell Biol, 1995. **15**(4): p. 2135-44.
24. Peyssonnaux, C., et al., *Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs).* J Clin Invest, 2007. **117**(7): p. 1926-32.
25. D'Andrea, A.D. and L.I. Zon, *Erythropoietin receptor. Subunit structure and activation.* J Clin Invest, 1990. **86**(3): p. 681-7.
26. Kuhrt, D. and D.M. Wojchowski, *Emerging EPO and EPO receptor regulators and signal transducers.* Blood, 2015. **125**(23): p. 3536-41.
27. Verma, R., et al., *RHEX, a novel regulator of human erythroid progenitor cell expansion and erythroblast development.* J Exp Med, 2014. **211**(9): p. 1715-22.
28. Koulnis, M., et al., *Contrasting dynamic responses in vivo of the Bcl-xL and Bim erythropoietic survival pathways.* Blood, 2012. **119**(5): p. 1228-39.
29. Rhodes, M.M., et al., *Bcl-x(L) prevents apoptosis of late-stage erythroblasts but does not mediate the antiapoptotic effect of erythropoietin.* Blood, 2005. **106**(5): p. 1857-63.
30. Ingley, E., *Integrating novel signaling pathways involved in erythropoiesis.* IUBMB Life, 2012. **64**(5): p. 402-10.
31. Fleming, R.E. and P. Ponka, *Iron overload in human disease.* N Engl J Med, 2012. **366**(4): p. 348-59.
32. Huang, H., et al., *Contribution of STAT3 and SMAD4 pathways to the regulation of hepcidin by opposing stimuli.* Blood, 2009. **113**(15): p. 3593-9.
33. Tanno, T., et al., *High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin.* Nat Med, 2007. **13**(9): p. 1096-101.
34. Jay, V., *Richard Bright--physician extraordinaire.* Arch Pathol Lab Med, 2000. **124**(9): p. 1262-3.
35. Plzak, L.F., et al., *Demonstration of stimulation of erythropoiesis by plasma from anemic rats using Fe59.* J Lab Clin Med, 1955. **46**(5): p. 671-8.
36. Jacobson, L.O., et al., *Role of the kidney in erythropoiesis. 1957.* J Am Soc Nephrol, 2000. **11**(3): p. 589-90; discussion 589-91.

37. Jacobs, K., et al., *Isolation and characterization of genomic and cDNA clones of human erythropoietin.* Nature, 1985. **313**(6005): p. 806-10.
38. Miyake, T., C.K. Kung, and E. Goldwasser, *Purification of human erythropoietin.* J Biol Chem, 1977. **252**(15): p. 5558-64.
39. Babitt, J.L. and H.Y. Lin, *Mechanisms of anemia in CKD.* J Am Soc Nephrol, 2012. **23**(10): p. 1631-4.
40. Stenvinkel, P., et al., *Inflammation and outcome in end-stage renal failure: does female gender constitute a survival advantage?* Kidney Int, 2002. **62**(5): p. 1791-8.
41. Pecoits-Filho, R., et al., *Associations between circulating inflammatory markers and residual renal function in CRF patients.* Am J Kidney Dis, 2003. **41**(6): p. 1212-8.
42. Kimmel, P.L., et al., *Immunologic function and survival in hemodialysis patients.* Kidney Int, 1998. **54**(1): p. 236-44.
43. Hasper, D., et al., *Systemic inflammation in patients with heart failure.* Eur Heart J, 1998. **19**(5): p. 761-5.
44. Shi, K., et al., *Gut bacterial translocation may aggravate microinflammation in hemodialysis patients.* Dig Dis Sci, 2014. **59**(9): p. 2109-17.
45. Spittle, M.A., et al., *Oxidative stress and inflammation in hemodialysis patients.* Am J Kidney Dis, 2001. **38**(6): p. 1408-13.
46. Banerjee, T., et al., *Vascular access type, inflammatory markers, and mortality in incident hemodialysis patients: the Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (CHOICE) Study.* Am J Kidney Dis, 2014. **64**(6): p. 954-61.
47. Dukkipati, R., et al., *Association of vascular access type with inflammatory marker levels in maintenance hemodialysis patients.* Semin Dial, 2014. **27**(4): p. 415-23.
48. Ayus, J.C. and D. Sheikh-Hamad, *Silent infection in clotted hemodialysis access grafts.* J Am Soc Nephrol, 1998. **9**(7): p. 1314-7.
49. Lopez-Gomez, J.M., et al., *Presence of a failed kidney transplant in patients who are on hemodialysis is associated with chronic inflammatory state and erythropoietin resistance.* J Am Soc Nephrol, 2004. **15**(9): p. 2494-501.
50. Honda, H., et al., *Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD.* Am J Kidney Dis, 2006. **47**(1): p. 139-48.
51. Panichi, V., et al., *Anaemia and resistance to erythropoiesis-stimulating agents as prognostic factors in haemodialysis patients: results from the RISCAVID study.* Nephrol Dial Transplant, 2011. **26**(8): p. 2641-8.
52. Zhang, Y., et al., *Epoetin requirements predict mortality in hemodialysis patients.* Am J Kidney Dis, 2004. **44**(5): p. 866-76.
53. Kalantar-Zadeh, K., et al., *Effect of malnutrition-inflammation complex syndrome on EPO hyporesponsiveness in maintenance hemodialysis patients.* Am J Kidney Dis, 2003. **42**(4): p. 761-73.
54. Macdougall, I.C. and A.C. Cooper, *Erythropoietin resistance: the role of inflammation and pro-inflammatory cytokines.* Nephrol Dial Transplant, 2002. **17 Suppl 11**: p. 39-43.

55. Macdougall, I.C. and A.C. Cooper, *Hyporesponsiveness to erythropoietic therapy due to chronic inflammation.* Eur J Clin Invest, 2005. **35 Suppl 3**: p. 32-5.
56. Cooper, A.C., et al., *Increased expression of erythropoiesis inhibiting cytokines (IFN-gamma, TNF-alpha, IL-10, and IL-13) by T cells in patients exhibiting a poor response to erythropoietin therapy.* J Am Soc Nephrol, 2003. **14**(7): p. 1776-84.
57. Goicoechea, M., et al., *Role of cytokines in the response to erythropoietin in hemodialysis patients.* Kidney Int, 1998. **54**(4): p. 1337-43.
58. Won, H.S., et al., *IL-6 is an independent risk factor for resistance to erythropoiesis-stimulating agents in hemodialysis patients without iron deficiency.* Hemodial Int, 2012. **16**(1): p. 31-7.
59. Kanbay, M., et al., *Erythropoiesis stimulatory agent- resistant anemia in dialysis patients: review of causes and management.* Blood Purif, 2010. **29**(1): p. 1-12.
60. Faquin, W.C., T.J. Schneider, and M.A. Goldberg, *Effect of inflammatory cytokines on hypoxia-induced erythropoietin production.* Blood, 1992. **79**(8): p. 1987-94.
61. Babitt, J.L. and H.Y. Lin, *Molecular mechanisms of hepcidin regulation: implications for the anemia of CKD.* Am J Kidney Dis, 2010. **55**(4): p. 726-41.
62. Allen, D.A., et al., *Inhibition of CFU-E colony formation in uremic patients with inflammatory disease: role of IFN-gamma and TNF-alpha.* J Investig Med, 1999. **47**(5): p. 204-11.
63. Chung, S., et al., *Relationship between erythropoietin resistance index and left ventricular mass and function and cardiovascular events in patients on chronic hemodialysis.* Hemodial Int, 2012. **16**(2): p. 181-7.
64. Badve, S.V., et al., *Interventions for erythropoietin-resistant anaemia in dialysis patients.* Cochrane Database Syst Rev, 2013. **8**: p. CD006861.
65. Bhatt, D.L., *Anti-inflammatory agents and antioxidants as a possible "third great wave" in cardiovascular secondary prevention.* Am J Cardiol, 2008. **101**(10A): p. 4D-13D.
66. Libby, P., P.M. Ridker, and A. Maseri, *Inflammation and atherosclerosis.* Circulation, 2002. **105**(9): p. 1135-43.
67. Libby, P., et al., *Inflammation in atherosclerosis: from pathophysiology to practice.* J Am Coll Cardiol, 2009. **54**(23): p. 2129-38.
68. Zimmermann, J., et al., *Inflammation enhances cardiovascular risk and mortality in hemodialysis patients.* Kidney Int, 1999. **55**(2): p. 648-58.
69. Gummer, J., et al., *Association between serum hepcidin-25 and primary resistance to erythropoiesis-stimulating agents in chronic kidney disease: a secondary analysis of the HERO trial.* Nephrology (Carlton), 2017. **22**(7): p. 548-554.
70. Chonchol, M., et al., *Chronic kidney disease is associated with angiographic coronary artery disease.* Am J Nephrol, 2008. **28**(2): p. 354-60.
71. Kilickesmez, K.O., et al., *Chronic kidney disease as a predictor of coronary lesion morphology.* Angiology, 2010. **61**(4): p. 344-9.
72. Nakano, T., et al., *Association of kidney function with coronary atherosclerosis and calcification in autopsy samples from Japanese elders: the Hisayama study.* Am J Kidney Dis, 2010. **55**(1): p. 21-30.

73. Weiner, D.E., et al., *The Framingham predictive instrument in chronic kidney disease.* J Am Coll Cardiol, 2007. **50**(3): p. 217-24.
74. Descamps-Latscha, B., et al., *Advanced oxidation protein products as risk factors for atherosclerotic cardiovascular events in nondiabetic predialysis patients.* Am J Kidney Dis, 2005. **45**(1): p. 39-47.
75. Stenvinkel, P., O. Heimburger, and T. Jogestrand, *Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dialysis patients: association with Chlamydia pneumoniae seropositivity.* Am J Kidney Dis, 2002. **39**(2): p. 274-82.
76. Foley, R.N., et al., *Septicemia in the United States dialysis population, 1991 to 1999.* J Am Soc Nephrol, 2004. **15**(4): p. 1038-45.
77. deFilippi, C., et al., *Cardiac troponin T and C-reactive protein for predicting prognosis, coronary atherosclerosis, and cardiomyopathy in patients undergoing long-term hemodialysis.* JAMA, 2003. **290**(3): p. 353-9.
78. Owen, W.F. and E.G. Lowrie, *C-reactive protein as an outcome predictor for maintenance hemodialysis patients.* Kidney Int, 1998. **54**(2): p. 627-36.
79. Herzog, C.A., et al., *Cause-specific mortality of dialysis patients after coronary revascularization: why don't dialysis patients have better survival after coronary intervention?* Nephrol Dial Transplant, 2008. **23**(8): p. 2629-33.
80. Foley, R.N., et al., *Clinical and echocardiographic disease in patients starting end-stage renal disease therapy.* Kidney Int, 1995. **47**(1): p. 186-92.
81. Clinton, S.K., et al., *Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis.* Am J Pathol, 1992. **140**(2): p. 301-16.
82. Gupta, S., et al., *IFN-gamma potentiates atherosclerosis in ApoE knock-out mice.* J Clin Invest, 1997. **99**(11): p. 2752-61.
83. Woollard, K.J. and F. Geissmann, *Monocytes in atherosclerosis: subsets and functions.* Nat Rev Cardiol, 2010. **7**(2): p. 77-86.
84. Tabas, I., K.J. Williams, and J. Boren, *Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications.* Circulation, 2007. **116**(16): p. 1832-44.
85. Libby, P., P.M. Ridker, and G.K. Hansson, *Progress and challenges in translating the biology of atherosclerosis.* Nature, 2011. **473**(7347): p. 317-25.
86. Hansson, G.K., *Inflammation, atherosclerosis, and coronary artery disease.* N Engl J Med, 2005. **352**(16): p. 1685-95.
87. Lee, S.J., et al., *Reversal of vascular 18F-FDG uptake with plasma high-density lipoprotein elevation by atherogenic risk reduction.* J Nucl Med, 2008. **49**(8): p. 1277-82.
88. Kausz, A.T., et al., *Intractable anemia among hemodialysis patients: a sign of suboptimal management or a marker of disease?* Am J Kidney Dis, 2005. **45**(1): p. 136-47.
89. Le Guludec, D., et al., *Present and future of clinical cardiovascular PET imaging in Europe--a position statement by the European Council of Nuclear Cardiology (ECNC).* Eur J Nucl Med Mol Imaging, 2008. **35**(9): p. 1709-24.

90. Mochizuki, T., et al., *FDG uptake and glucose transporter subtype expressions in experimental tumor and inflammation models.* J Nucl Med, 2001. **42**(10): p. 1551-5.
91. Rudd, J.H., et al., *Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography.* Circulation, 2002. **105**(23): p. 2708-11.
92. Tahara, N., et al., *Simvastatin attenuates plaque inflammation: evaluation by fluorodeoxyglucose positron emission tomography.* J Am Coll Cardiol, 2006. **48**(9): p. 1825-31.
93. Tarkin, J.M., F.R. Joshi, and J.H. Rudd, *PET imaging of inflammation in atherosclerosis.* Nat Rev Cardiol, 2014. **11**(8): p. 443-57.
94. Evans, N.R., et al., *PET Imaging of Atherosclerotic Disease: Advancing Plaque Assessment from Anatomy to Pathophysiology.* Curr Atheroscler Rep, 2016. **18**(6): p. 30.
95. Figueroa, A.L., et al., *Measurement of arterial activity on routine FDG PET/CT images improves prediction of risk of future CV events.* JACC Cardiovasc Imaging, 2013. **6**(12): p. 1250-9.
96. Ishii, H., et al., *Comparison of atorvastatin 5 and 20 mg/d for reducing F-18 fluorodeoxyglucose uptake in atherosclerotic plaques on positron emission tomography/computed tomography: a randomized, investigator-blinded, open-label, 6-month study in Japanese adults scheduled for percutaneous coronary intervention.* Clin Ther, 2010. **32**(14): p. 2337-47.
97. Tawakol, A., et al., *Intensification of statin therapy results in a rapid reduction in atherosclerotic inflammation: results of a multicenter fluorodeoxyglucose-positron emission tomography/computed tomography feasibility study.* J Am Coll Cardiol, 2013. **62**(10): p. 909-17.
98. Takx, R.A., et al., *Increased arterial inflammation in individuals with stage 3 chronic kidney disease.* Eur J Nucl Med Mol Imaging, 2015.
99. Moody, W.E., et al., *Arterial disease in chronic kidney disease.* Heart, 2013. **99**(6): p. 365-72.
100. Niiranen, T.J., et al., *Relative Contributions of Arterial Stiffness and Hypertension to Cardiovascular Disease: The Framingham Heart Study.* J Am Heart Assoc, 2016. **5**(11).
101. Redheuil, A., et al., *Reduced ascending aortic strain and distensibility: earliest manifestations of vascular aging in humans.* Hypertension, 2010. **55**(2): p. 319-26.
102. van der Meer, R.W., et al., *Magnetic resonance assessment of aortic pulse wave velocity, aortic distensibility, and cardiac function in uncomplicated type 2 diabetes mellitus.* J Cardiovasc Magn Reson, 2007. **9**(4): p. 645-51.
103. Peyster, E., et al., *Inflammation and Arterial Stiffness in Chronic Kidney Disease: Findings From the CRIC Study.* Am J Hypertens, 2017. **30**(4): p. 400-408.
104. Maki-Petaja, K.M., et al., *Anti-tumor necrosis factor-alpha therapy reduces aortic inflammation and stiffness in patients with rheumatoid arthritis.* Circulation, 2012. **126**(21): p. 2473-80.

105. Maki-Petaja, K.M., et al., *Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy.* Circulation, 2006. **114**(11): p. 1185-92.
106. Hashimoto, J. and M.F. O'Rourke, *Inflammation and Arterial Stiffness in Chronic Kidney Disease: Cause or Consequence?* Am J Hypertens, 2017. **30**(4): p. 350-352.
107. Adenwalla, S.F., et al., *The importance of accurate measurement of aortic stiffness in patients with chronic kidney disease and end-stage renal disease.* Clin Kidney J, 2017. **10**(4): p. 503-515.
108. Chue, C.D., et al., *Arterial stiffness in chronic kidney disease: causes and consequences.* Heart, 2010. **96**(11): p. 817-23.
109. Blacher, J., et al., *Impact of aortic stiffness on survival in end-stage renal disease.* Circulation, 1999. **99**(18): p. 2434-9.
110. Vlachopoulos, C., et al., *Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis.* Eur Heart J, 2010. **31**(15): p. 1865-71.
111. Maroules, C.D., et al., *Cardiovascular outcome associations among cardiovascular magnetic resonance measures of arterial stiffness: the Dallas heart study.* J Cardiovasc Magn Reson, 2014. **16**: p. 33.
112. Ferrari, P., et al., *Pentoxifylline improves haemoglobin and interleukin-6 levels in chronic kidney disease.* Nephrology (Carlton), 2010. **15**(3): p. 344-9.
113. Navarro, J.F., et al., *Effects of pentoxifylline on the haematologic status in anaemic patients with advanced renal failure.* Scand J Urol Nephrol, 1999. **33**(2): p. 121-5.
114. Gonzalez-Espinoza, L., et al., *Pentoxifylline decreases serum levels of tumor necrosis factor alpha, interleukin 6 and C-reactive protein in hemodialysis patients: results of a randomized double-blind, controlled clinical trial.* Nephrol Dial Transplant, 2012. **27**(5): p. 2023-8.
115. Deree, J., et al., *Insights into the regulation of TNF-alpha production in human mononuclear cells: the effects of non-specific phosphodiesterase inhibition.* Clinics (Sao Paulo), 2008. **63**(3): p. 321-8.
116. Kiely, P.D., K.M. Gillespie, and D.B. Oliveira, *Oxpentifylline inhibits tumor necrosis factor-alpha mRNA transcription and protects against arthritis in mercuric chloride-treated brown Norway rats.* Eur J Immunol, 1995. **25**(10): p. 2899-906.
117. Antunes, S.A., et al., *Pentoxifylline does not alter the concentration of hepcidin in chronic kidney disease patients undergoing hemodialysis.* Int J Artif Organs, 2014. **37**(7): p. 521-8.
118. *IV. NKF-K/DOQI Clinical Practice Guidelines for Anemia of Chronic Kidney Disease: update 2000.* Am J Kidney Dis, 2001. **37**(1 Suppl 1): p. S182-238.
119. Locatelli, F., et al., *Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure.* Nephrol Dial Transplant, 2004. **19 Suppl 2**: p. ii1-47.
120. *Chapter 1: Diagnosis and evaluation of anemia in CKD.* Kidney Int Suppl (2011), 2012. **2**(4): p. 288-291.
121. Panichi, V., et al., *Anaemia and resistance to erythropoiesis-stimulating agents as prognostic factors in haemodialysis patients: results from the RISCAVID study.* Nephrol Dial Transplant, 2011. **26**(8): p. 2641-8.

122. Badve, S.V., et al., *Interventions for erythropoietin-resistant anaemia in dialysis patients.* Cochrane Database Syst Rev, 2013(8): p. CD006861.
123. Johnson, D.W., et al., *A randomized, placebo-controlled trial of pentoxifylline on erythropoiesis-stimulating agent hyporesponsiveness in anemic patients with CKD: the Handling Erythropoietin Resistance With Oxpentifylline (HERO) trial.* Am J Kidney Dis, 2015. **65**(1): p. 49-57.
124. Icardi, A., et al., *Renal anaemia and EPO hyporesponsiveness associated with vitamin D deficiency: the potential role of inflammation.* Nephrol Dial Transplant, 2013. **28**(7): p. 1672-9.
125. Samouilidou, E., et al., *Serum hepcidin levels are associated with serum triglycerides and interleukin-6 concentrations in patients with end-stage renal disease.* Ther Apher Dial, 2014. **18**(3): p. 279-83.
126. Mortazavi, M., et al., *Role of pentoxifylline in treatment of anemic patients suffering chronic hemodialysis: a randomized clinical trial.* Med Arh, 2012. **66**(2): p. 84-6.
127. Shahbazian, H., et al., *Administration of pentoxifylline to improve anemia of hemodialysis patients.* J Renal Inj Prev, 2017. **6**(1): p. 61-64.
128. Thursz, M.R., et al., *Prednisolone or Pentoxifylline for Alcoholic Hepatitis.* N Engl J Med, 2015. **373**(3): p. 282-3.
129. Navarro-Gonzalez, J.F., et al., *Effect of pentoxifylline on renal function and urinary albumin excretion in patients with diabetic kidney disease: the PREDIAN trial.* J Am Soc Nephrol, 2015. **26**(1): p. 220-9.
130. Dember, L.M., et al., *Pragmatic Trials in Maintenance Dialysis: Perspectives from the Kidney Health Initiative.* J Am Soc Nephrol, 2016. **27**(10): p. 2955-2963.
131. Jha, V., et al., *Chronic kidney disease: global dimension and perspectives.* Lancet, 2013. **382**(9888): p. 260-72.
132. Foley, R.N., P.S. Parfrey, and M.J. Sarnak, *Clinical epidemiology of cardiovascular disease in chronic renal disease.* Am J Kidney Dis, 1998. **32**(5 Suppl 3): p. S112-9.
133. Wanner, C., et al., *Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis.* N Engl J Med, 2005. **353**(3): p. 238-48.
134. Holdaas, H., et al., *Rosuvastatin in diabetic hemodialysis patients.* J Am Soc Nephrol, 2011. **22**(7): p. 1335-41.
135. Wanner, C. and V. Krane, *Sunrise of statins after AURORA and 4D?* J Am Soc Nephrol, 2011. **22**(7): p. 1184-6.
136. Baigent, C., et al., *The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial.* Lancet, 2011. **377**(9784): p. 2181-92.
137. Zimmerli, L.U., et al., *Vascular function in patients with end-stage renal disease and/or coronary artery disease: a cardiac magnetic resonance imaging study.* Kidney Int, 2007. **71**(1): p. 68-73.
138. Guerin, A.P., et al., *Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure.* Circulation, 2001. **103**(7): p. 987-92.
139. Sarafidis, P.A., et al., *Ambulatory Pulse Wave Velocity Is a Stronger Predictor of Cardiovascular Events and All-Cause Mortality Than Office*

and Ambulatory Blood Pressure in Hemodialysis Patients. Hypertension, 2017. **70**(1): p. 148-157.
140. Pietri, P., et al., *Relationship between low-grade inflammation and arterial stiffness in patients with essential hypertension.* J Hypertens, 2006. **24**(11): p. 2231-8.
141. Mahmud, A. and J. Feely, *Arterial stiffness is related to systemic inflammation in essential hypertension.* Hypertension, 2005. **46**(5): p. 1118-22.
142. Shao, J.S., et al., *Inflammation and the osteogenic regulation of vascular calcification: a review and perspective.* Hypertension, 2010. **55**(3): p. 579-92.
143. Metry, G., et al., *Low serum fetuin-A concentration predicts poor outcome only in the presence of inflammation in prevalent haemodialysis patients.* Eur J Clin Invest, 2008. **38**(11): p. 804-11.
144. Goicoechea, M., et al., *Effects of pentoxifylline on inflammatory parameters in chronic kidney disease patients: a randomized trial.* J Nephrol, 2012. **25**(6): p. 969-75.
145. Mohammadpour, A.H., et al., *Evaluation of the effect of pentoxifylline on erythropoietin-resistant anemia in hemodialysis patients.* Saudi J Kidney Dis Transpl, 2014. **25**(1): p. 73-8.
146. Navarro, J.F., et al., *Urinary protein excretion and serum tumor necrosis factor in diabetic patients with advanced renal failure: effects of pentoxifylline administration.* Am J Kidney Dis, 1999. **33**(3): p. 458-63.
147. Chen, Y.M., et al., *Pentoxifylline ameliorates proteinuria through suppression of renal monocyte chemoattractant protein-1 in patients with proteinuric primary glomerular diseases.* Kidney Int, 2006. **69**(8): p. 1410-5.
148. Lin, S.L., et al., *Pentoxifylline attenuated the renal disease progression in rats with remnant kidney.* J Am Soc Nephrol, 2002. **13**(12): p. 2916-29.
149. Lin, S.L., et al., *Pentoxifylline attenuates tubulointerstitial fibrosis by blocking Smad3/4-activated transcription and profibrogenic effects of connective tissue growth factor.* J Am Soc Nephrol, 2005. **16**(9): p. 2702-13.
150. Navarro-Gonzalez, J.F., et al., *Effects of Pentoxifylline on Soluble Klotho Concentrations and Renal Tubular Cell Expression in Diabetic Kidney Disease.* Diabetes Care, 2018. **41**(8): p. 1817-1820.
151. Jiang, X., et al., *Effect of pentoxifylline in proteinuric chronic kidney disease: a systematic review and meta-analysis.* J Nephrol, 2016. **29**(5): p. 653-62.
152. Leporini, C., et al., *Effect of pentoxifylline on renal outcomes in chronic kidney disease patients: A systematic review and meta-analysis.* Pharmacol Res, 2016. **107**: p. 315-332.
153. Brenner, B.M., et al., *Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy.* N Engl J Med, 2001. **345**(12): p. 861-9.
154. Ruggenenti, P., et al., *Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria.* Lancet, 1999. **354**(9176): p. 359-64.
155. Hou, F.F., et al., *Renoprotection of Optimal Antiproteinuric Doses (ROAD) Study: a randomized controlled study of benazepril and*

losartan in chronic renal insufficiency. J Am Soc Nephrol, 2007. **18**(6): p. 1889-98.
156. Kuo, K.L., et al., *Add-on Protective Effect of Pentoxifylline in Advanced Chronic Kidney Disease Treated with Renin-Angiotensin-Aldosterone System Blockade - A Nationwide Database Analysis.* Sci Rep, 2015. **5**: p. 17150.
157. Wu, P.C., et al., *Pentoxifylline Decreases Dialysis Risk in Patients With Advanced Chronic Kidney Disease.* Clin Pharmacol Ther, 2015. **98**(4): p. 442-9.
158. Liu, D., et al., *Pentoxifylline plus ACEIs/ARBs for proteinuria and kidney function in chronic kidney disease: a meta-analysis.* J Int Med Res, 2017. **45**(2): p. 383-398.
159. Shafi, T. and E. Guallar, *Mapping Progress in Reducing Cardiovascular Risk with Kidney Disease: Sudden Cardiac Death.* Clin J Am Soc Nephrol, 2018. **13**(9): p. 1429-1431.
160. McCarty, M.F., J.H. O'Keefe, and J.J. DiNicolantonio, *Pentoxifylline for vascular health: a brief review of the literature.* Open Heart, 2016. **3**(1): p. e000365.

Figure 7.1 Image adapted from

PET Imaging of Atherosclerotic Disease: Advancing Plaque Assessment from Anatomy to Pathophysiology.
Evans NR, Tarkin JM, Chowdhury MM, Warburton EA, Rudd JH.
Curr Atheroscler Rep. 2016 Jun;18(6):30. doi: 10.1007/s11883-016-0584-3. Review.

Figure 7.2 Image adapted from

Relationship Between Measures of Adiposity, Arterial Inflammation, and Subsequent Cardiovascular Events.
Figueroa AL, Takx RA, MacNabb MH, Abdelbaky A, Lavender ZR, Kaplan RS, Truong QA, Lo J, Ghoshhajra BB, Grinspoon SK, Hoffmann U, Tawakol A.
Circ Cardiovasc Imaging. 2016 Apr;9(4):e004043. doi: 10.1161/CIRCIMAGING.115.004043
2013. **6**(12): p. 1250-9. Permissions requested

Appendix : Study Protocols

15.1 Protocol: PEAR study

15.2 Protocol: PEAR study : Experimental outcomes

15.3 Study consent form

15.4 Ethics committee and MHRA approval

15.1 Protocol: PEAR Study

TITLE OF THE PROTOCOL:

A Single-Centre randomized placebo controlled, double blinded study of Pentoxifylline in End-Stage Renal Disease Patients with Erythropoeitin resistance

Short title/Acronym: PEntoxifylline in Anaemia Resistant to erythropoietin (PEAR)

Sponsor: Barts Health NHS Trust

Representative of the Sponsor:
Sally Burtles
Director of Research Services and Business Development
Joint Research Management Office
5 Walden Street
London
E1 2EF
Phone: 020 7882 7260
Email: sponsorsrep@bartshealth.nhs.uk

REC reference: **12/LO/1635**

EudraCT reference: **2011-006168-30**

Chief Investigator Agreement Page

The clinical study as detailed within this research protocol **(**Protocol PEAR_20March2013_v8-1**)**, or any subsequent amendments, involves the use of an investigational medicinal product and will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Chief Investigator Name:

Prof Magdi M Yaqoob

Chief Investigator Site:

Barts Health NHS Trust

Signature and Date:

Statistician Agreement Page

The clinical study as detailed within this research protocol **(**Protocol PEAR_20March2013_v8-1**)**, or any subsequent amendments, involves the use of an investigational medicinal product and will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Statistician Name: Dr Stanley FAN

Site: Barts Health NHS Trust

Signature and Date:

Investigator site: Barts Health NHS Trust

Responsible investigator	Prof Magdi Yaqoob
Co-investigator(s)	Dr S Fan, Dr F Pugliese, Dr C Davies, Dr S Petersen, Prof C Thiemermann

Laboratory sites (describe for each assay)

Assay	Laboratory (name / adress)	Contact person
Chemistry	Clinical Biochemistry Dr Sally Benton Consultant Clinical Scientist Barts Health NHS Trust 020 7377 7000 Extn 60386	
Chemistry (hs-CRP) and storage of serum		
Haematology		
Cytokine Assays	• Lab Manager: Dr Steve Harwood • Department of Translational Medicine and Therapeutics, John Vane Building, William Harvey Research Institute, Charterhouse quare, London EC1 Telephone number: 02078822121	
DNA telomere length		

Pharmacy	Contact person
Pharmacy Unit, Barts Health NHS Trust	Ms Josephine Falade

STUDY SUMMARY/SYNOPSIS

TITLE	A Single-Centre randomized placebo controlled, double blinded study of Pentoxifylline in End-Stage Renal Disease Patients with Erythropoeitin resistance
SHORT TITLE	PEntoxifylline in Anaemia Resistant to erythropoietin (PEAR)
Protocol Version Number and Date	Protocol PEAR_29th Oct.2013_v 10-1
Methodology	A Single-Centre randomized placebo controlled, double blinded study
Study Duration	24 months
Study Centre	Barts Health NHS Trust
Rationale	Unfortunately, a considerable proportion of ESRD patients exhibit a suboptimal haematologic response to ESA. In some patients, this has been attributed to elevated levels of inflammatory cytokines including TNF-α and IFN-γ. Pentoxifylline specifically inhibits T-cell production of TNF-α and IFN-γ and in a small uncontrolled study suggested this drug was a useful adjunct for patients with ESA resistance.
Objectives	To study the effects Pentoxifylline in ESA resistant ESRD patients on haemodialysis *Endpoints*: The primary study end point is the ESA requirement relative to the Hb level. Secondary endpoints include: • Safety analysis • Hb values and ESA doses after 6 months of treatment. • Blood sampling will be performed at the start of each hemodialysis session every month. • Cardiovascular imaging will be performed at baseline and at 6 months. The effect IMP has on the following cardiovascular parameters will be examined: o mean target-to-background ratio across a substantial portion of artery (typically aorta, supraaortic vessels and femoral arteries) using 18-FDG-PET (time-of-

	flight). This will allow us to assess whether IMP has an anti-inflamatory effect on the blood vessels o vascular stiffness measures (e.g. aortic distensibility at three levels of aorta (mmHg-1), pulse wave velocity (m/s) and carotid distensibility (mmHg-1))using magnetic resonance (MR) imaging. o Measures of systolic and diastolic function, using cardiac MR (CMR) tagging techniques (strain (%) and strain-rate (s-1))
Phase of the Trial	II
Number of Subjects/Patients	100.
Main Inclusion Criteria	Inclusion Criteria • Be able to read and understand the written consent form, complete study-related procedures, and communicate with the study staff; • Willing to comply with study restrictions; • Between 18 and 85 years of age (inclusive). • Diagnosis of clinically stable ESRD, as determined by the investigator; • Requiring regular dialysis therapy for at least 12 weeks prior to first administration of study agent; • Receiving treatment with IV or SC erythropoietin receptor agonist at least weekly (ie exclude Micera or other ESAs given fortnightly or monthly) for a minimum of 8 weeks prior to administration of study agent, requiring doses to remedy EPO-resistance (requiring greater than or equal to 6000iu equivalent of EPO per week or if ESA resistance index is greater than or equal to 6.5iu /kg/wk/g Hb for equivalent EPO dose), with evidence of stable hemoglobin levels • Baseline hemoglobin values between 9.0 and 12.0 g/dL before entering the study; • CRP levels of ≥5 mg/L
Statistical Methodology and Analysis	We expect that the mean of the run-in and last 2 valid visits will be used for primary end-point analyses. Primary Endpoint Analysis: We shall be compare δEPO/kg requirement between the 2 groups using unpaired student t-test.

Glossary of Terms and Abbreviations

°□C	Degree Celsius
µg	Microgram
AE	Adverse Event
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AR	Adverse Reaction
AST	Aspartate aminotransferase
BHT	Barts Health Trust
BUN	Blood nitrogen urea
CK	Creatine kinase
CKD	Chronic Kidney Disease
CMR	Cardiac Magnetic Resonance Imaging
CRF	Case Report Form
(hs-)CRP	(high sensitive) C- reactive protein
CRF	Clinical Research Form
CSR	Clinical Study Report
CV	Cardiovascular
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
EC	European Community
ECG	Electrocardiogram
EMEA	European Medicines Agency
EOT	End of Trial
EPO	Erythropoietin
ESA	Erythropoietin Stimulating Agents
ESRD	End Stage Renal Disease
FBC	Full blood count
FDA	Food and Drug Administration
GCP	Good Clinical Practice
Glc	glucose
Hb	Haemoglobin
HD	Haemodialysis (inclhaemodiafiltration or haemofiltration)
hr(s)	Hour(s)
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
INF-γ	Interferon-γ
IP	Investigational Product
IRB	Institutional Review Board
ITT	Intention to Treat
iu	International units
iv	intravenous
kg	Kilogram
L	Litres
LFT	Liver Function Tests
MCH(C)	Mean Corpuscular Hemoglobin (Concentration)
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligrams

MHRA	Medicine Health Regulatory Authority
mL	Milliliter
NA	Not applicable
NCS	Not clinically significant
OTC	Over the counter (non-prescription medication)
PET-CT	Positron emission tomography – computed tomography
PIS	Patient Information Sheet
PTH	Parathyroid hormone
RBC	Red Blood Cell
R&D	Research and Development Department
RLH	Royal London Hospital
SAE	Serious Adverse Event
sc	Subcutaneously
SPC	Summary of Product Characteristic
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNF-α	Tumour Necrosis Factor-α
TMF	Trial Master file
Tsats	Transferrin saturation
U&E	Urea & Electrolytes
VS	Vital signs
WBC	White Blood Cell
WHO	World Health Organization

Contents

1 Introduction

1.1 Background

Pentoxifylline (used for twenty years in the treatment of peripheral and cerebral vascular diseases), has potent haemorrheological and anti-inflammatory properties (secondary to inhibition of phosphodiesterases) and (in 2 small, prospective, non-randomized phase 2a studies)improved haemoglobin levels in CKD patients with ESA-resistant anaemia.

The use of ESA in resistant patients is very expensive: Twenty % of the patients on dialysis have ESA-resistance, which is a surrogate marker for excessive, all-cause and CV mortality. There are no specific therapeutic strategies available to these patients. Moreover, the health economic consideration for the treatment of ESA-resistant patients with ever increasing amounts of ESAis very significant. In BLT, the cost for ESA alone for 250 ESA-resistant patients amounts to £ 2.5 Million per year which is the same cost as for the remaining 950 ESA-sensitive patients.

If the proposed strategy is successful in ESA-resistant dialysis patients, then it may also be employed in so-called ESA-sensitive' patients in order to lower their ESA-requirements (and to make them *'more sensitive'*).

1.2 Investigational Medicinal Product

Pentoxifylline may also have other pleiotropic effects that may be due to direct action or mediated by enhanced tissue-protective effects of ESA that has been well documented. We are particularly interested in any possible synergistic actions of the direct anti-inflammatory action of pentoxifylline and ESA that may be beneficial to the vascular abnormalities that are found in ESRD patients.

In fact, sudden cardiac and other cardiovascular events are the most common causes of death in ESRD patients. Surrogate markers of vascular abnormalities that have been found to correlate closely to morbidity and mortality include vascular calcification, pulse wave velocity and impaired vasodilation.

We therefore wish to examine if Pentoxifylline is beneficial in ESRD patients with evidence of ESA-resistance. We wish to examine if the treatment improves ESA response and if we can detect any improvements in biochemical &radiological surrogate markers of vascular health.

1.3 Preclinical Data

SmPC for Pentoxifylline states, "Nothing of clinical relevance" for pre-clinical safety data.

1.4 Clinical Data

Pentoxifyllineis a licenced treatment for peripheral vascular disease because of its potent hemorrheological properties. It also has anti-inflammatory properties, mediated viainhibition of phosphodiesterase.

Beneficial effects have been reported in idiopathic dilated cardiomyopathy, childhood type 1 diabetes, and systemic vasculitis. Modest clinical effects have also been observed in rheumatoid arthritis.

Two small, prospective, non-randomised studies have demonstrated that:

- Pentoxifyllinemay significantly improve haemoglobin levels in chronic kidney disease patients with ESA-resistant anaemia. Navarro et al treated 7 anaemic patients not on dialysis with oxpentifylline (oral 400 mg daily) for 6 months.
- Haemoglobin levels significantly increased from 9.9 ± 0.5 to 10.6 ± 0.6 g/dL ($p < 0.01$). Similarly, Cooper et al used the same dose for 4 months in 16 EPO-resistant anaemic dialysis patients. Among the 12 patients who completed the study, mean haemoglobin concentration increased from 9.5 ± 0.9 to 11.7 ± 1.0 g/L ($p = 0.0001$).

1.5 Rationale and Risks/Benefits

The following are side-effects listed in the SmPC for Pentoxifylline:

These adverse reactions have been reported in clinical trials or post-marketing. Frequencies are unknown.

System Organ Class	**Adverse Reaction**
Investigations	Transaminases increased
Cardiac disorders	Arrhythmia, Tachycardia, Angina Pectoris
Blood and lymphatic system disorders	Thrombocytopenia
Nervous system disorders	Dizziness, headache, meningitis aseptic*
Gastrointestinal disorders	Gastrointestinal disorder, Epigastric discomfort, Abdominal distension, Nausea, Vomiting, Diarrhoea
Skin and subcutaneous tissue disorders	Pruritus, Erythema, Urticaria, Hot flush
Vascular disorders	Haemorrhage**, Hypotension
Immune system disorders	Anaphylactic reactions, Anaphylactoid reaction, Angioedema
Hepatobiliary disorders	Cholestasis
Psychiatric disorders	Agitation, Sleep disorder
Respiratory disorders	Bronchospasm

Description of selected adverse reactions

* Reports of aseptic meningitis were predominantly in patients with underlying connective tissue disorders

** A few very rare events of bleeding (e.g. skin, mucosa) have been reported in patients treated with Trental with and without anticoagulants or platelet aggregation inhibitors. The serious cases are predominantly concentrated in the gastrointestinal, genitourinary, multiple site and surgical

wound areas and are associated with bleeding risk factors. A causal relationship between Trental therapy and bleeding has not been established. Thrombocytopenia has occurred in isolated cases.

2 Trial Objectives and Design

2.1 Trial Objectives

To study the effects Pentoxifylline in ESA resistant ESRD patients on haemodialysis

Endpoints:
Primary study endpointis the ESA requirement relative to the Hb level.

Secondary endpoints include:

- Safety analysis
- Hb values and ESA doses after 6 months of treatment.
- Cardiovascular imaging will be performed at baseline and at 6 months. The effect IMP has on the following cardiovascular parameters will be examined:
    - mean target-to-background ratio across a substantial portion of artery (typically aorta, supraaortic vessels and femoral arteries) using 18-FDG-PET (time-of-flight). This will allow us to assess whether IMP has an anti-inflamatory effect on the blood vessels
    - vascular stiffness measures (e.g. aortic distensibility at three levels of aorta (mmHg-1), pulse wave velocity (m/s) and carotid distensibility (mmHg-1))using magnetic resonance (MR) imagin
    - Measures of systolic and diastolic function, using cardiac MR (CMR) tagging techniques (strain (%) and strain-rate (s-1))

2.2 Trial Design

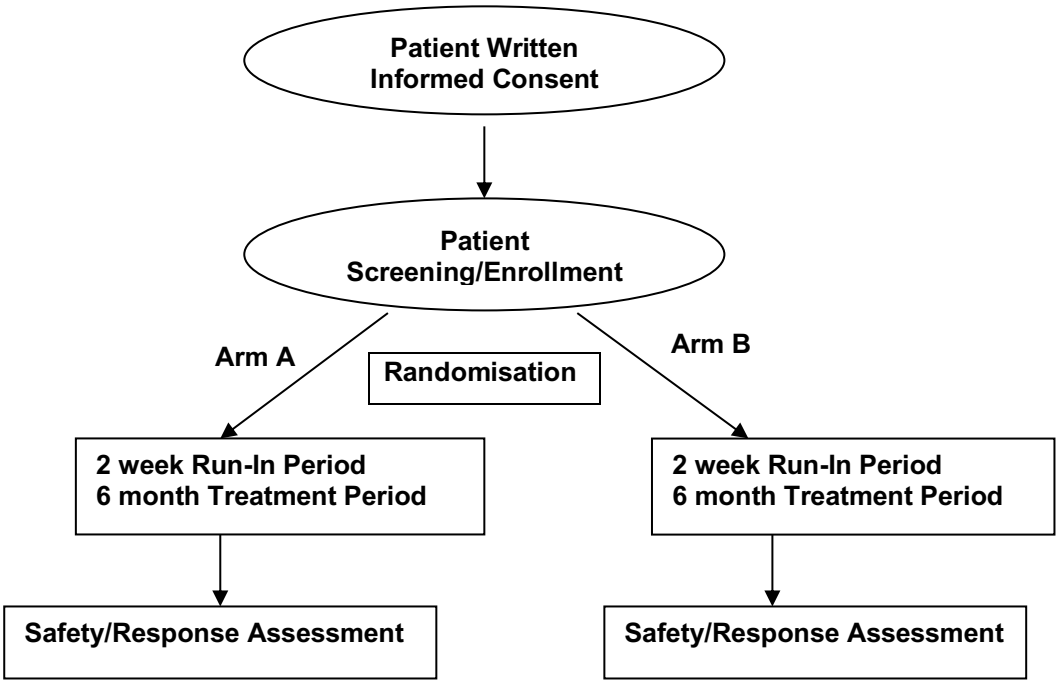
Study Design: This is a single-centre, randomised double blinded placebo controlled trial of100 patients with ESRD on dialysis. Patients will receive either Pentoxifylline 400mg once a day or placebo for 6 months with a further 2 month follow-up after therapy is stopped. Randomisation will be stratified for diabetes mellitus status and ESA requirement (adjusted for Hb).

Run-in Period: All subjects will undergo a run-in period of 2 weeks so that baseline haemoglobin levels can be measured once every week (+/- 3 days) so that we will have 3 values prior to starting IMP or placebo.

Drugs and Dosages: Study drug will be given orally and at a dose of 400mg once a day. This dose was previously shown to be well-tolerated in ESRD patients and could suppress *ex-vivo* T-cell generation of TNF-α and IFN-γ.

The dose of ESA for each patient will remain unchanged unless consecutive Hb values are <10.0 or >12g/dl (or for clinical indications). This reflects the Local Barts Health NHS Trust Renal Protocol for managing Hb / ESA dose. Intravenous iron supplementation will be given in accordance with the Local Barts Health NHS Trust Renal protocol. These protocols are listed in section 5.

2.3 Study Scheme Diagram


Patient Written Informed Consent
Patient Screening/Enrollment
Arm A
Randomisation
Arm B
2 week Run-In Period
6 month Treatment Period
2 week Run-In Period
6 month Treatment Period
Safety/Response Assessment
Safety/Response Assessment

3 Subject Selection

3.1 Number of Subjects and Subject Selection

We shall aim to recruit and treat a maximum of 100 patients (in each group) with end-stage renal disease established on dialysis. Although the power calculation below suggests we only require 30 patients (in each group) to reach the end of the 6 month treatment to achieve the statistical power detailed below, there is a very high drop out rate because of death and transplantation. We therefore do not expect to recruit more than 100 patients/group and it is possible that 30/group patients will be sufficient if drop-out rates are lower than expected.

Patients with ESA resistance defined as receiving treatment with IV or SC erythropoietin receptor agonist at least weekly (ie exclude Micera or other ESAs given fortnightly or monthly)for a minimum of 8 weeks prior to enrolment, requiring greater or equal to 6000 IU equivalent of EPO per week to achieve stable hemoglobin levels

All subjects will be screened for eligibility within 28 days before enrollment, based upon data obtained from the most recent clinical visit(s). The population will consist of patients of either gender, aged 18-85 years (inclusive).

3.1.1 Power calculation

From internal audit data on ESA usage, we assume the average dose of ESA will be 12000iu/week (with SD=500)
If the placebo group have Hb=10g/dl, the ESA/Hb ratio = 1200

The study by Cooper et al showed that 4 months treatment increased Hb levels by 25%.
We wish to power this study to demonstrate that IMP will increase ESA response by 5%

If we assumed the SD for ESA/Hb = 100, this will mean that for an alpha level of 5% (95%CI) and a beta level of 5% (statistical power of 95%) we shall need 30 patients in each group. We assume that there will be a 50% drop out (transplantation and patient withdrawal) so we plan to recruit no more than 100 patients/group

3.2 Inclusion Criteria

Eligibility for study participation will be based upon data obtained from the most recent clinical visit(s). In addition, recent medical history will be obtained prior to enrolment. To be eligible for study participation, subjects must meet all of the following criteria:

- Be able to read and understand the written consent form (with the help of a translator if necessary), complete study-related procedures, and communicate with the study staff;
- Willing to comply with study restrictions;
- Between 18 and 85 years of age (inclusive).
- Diagnosis of clinically stable ESRD, as determined by the investigator;
- Requiring regular dialysis therapy for at least 12 weeks prior to first administration of study agent;
- Last hemoglobin value at time of consent between 9.0 and 12.0 g/dL before entering the study;

- Receiving treatment with IV or SC erythropoietin receptor agonist at least weekly (ie exclude Micera or other ESAs given fortnightly or monthly) for a minimum of 8 weeks prior to administration of study agent, requiring doses to remedy EPO-resistance (requiring greater than or equal to 6000iu equivalent of EPO per week or if ESA resistance index is greater than or equal to 6.5iu /kg/wk/g Hb for equivalent EPO dose), with evidence of stable hemoglobin levels

- Serum folate and vitamin B12 levels are normal and T-sats>25% and / or ferritin > 200 mcg/l (last result prior to consent).

3.3 Exclusion Criteria

Eligibility for study participation will be based upon data obtained from the most recent clinical visit(s). In addition, recent medical history will be obtained prior to enrollment. If a subject displays any of the following criteria, he or she may not be enrolled in the study:

- Clinically relevant abnormal history of physical and mental health other than conditions related to chronic kidney disease of patient, as determined by medical history taking (as judged by the investigator);
- Clinically relevant abnormal laboratory results, ECG, vital signs, or physical findings other than conditions related to chronic kidney disease of patient (as judged by the investigator);
- Subject has uncontrolled hypertension (in the opinion of the clinician);
- Subject is unable to refrain from the use of disallowed concomitant medication from one week prior to the first study drug administration until follow-up assessments (see section 3.3);
- Participation in an investigational drug trial in the 3 months prior to administration of the initial dose of study drug
- Subject has undergone major surgery within 3 months prior to screening;
- Any other condition that in the opinion of the investigator would complicate or compromise the study (e.g. known haemoglobinopathy), or the well being of the subject.
- Females of child-bearing potential who are not willing to use contraception for the duration of the study.
- Females who are breast feeding.
- Subject is known hypersensitivity to the active constituent, pentoxifylline other methyl xanthines or any of the excipients.
- Subjects with recent cerebral haemorrhage, extensive retinal haemorrhage, acute myocardial infarction and severe cardiac arrhythmias
- Contraindications to magnetic resonance imaging (e.g. severe claustrophobia, pacemaker, defibrillators, etc.)
- Subjects who are on (or are due to start) immunosuppressive and anti-inflammatory drugs except Asprin at a dose of ≤ 300mg/d.

3.4 Criteria for Premature Withdrawal

Systematic noncompliance may culminate in a patient's withdrawal from the study.

We would perform the end-point tests (blood and radiology) if the patient agrees prior to formal withdrawal from the study.

The 1 month safety follow-up period (including blood tests) will also be performed if the patient agrees.

4 Investigational Medicinal Products (IMPs)

IMP: Overencapsulated Pentoxifylline,
Placebo: matching overencapsulated cellulose

4.1 Formulation of IMP

Capsules

4.2 IMP Supply

IMP and placebo are manufactured and supplied by Mawdsleys.

4.3 Prescription of IMP

A specifically designed Clinical trial prescription will be used for this study.

4.4 Preparation and Administration of IMP

The IMP will be given orally at a dose of Pentoxifyline 400mg once a day.

4.5 Packaging and Labelling of IMPs

A specifically designed Clinical trial label will be used for this study

4.6 Accountability/Receipt /Storage and Handling of IMP

The supply of IMP and Placebo will be shipped to Pharmacy at BHT.
Pharmacy will store and dispense study drugs for patients.
Drugs will be collected by Research Nurse or delegated investigator and handed to patients in study.
Drug accountability: All treatments for the study will be stored in the Department of Pharmacy of BHT until dispensing when it is required.

4.7 Dispensing of IMP

Pharmacy at BHT will dispense IMP

4.8 Prior and Concomitant Therapies

During the study the patients will continue all their regular therapy. Initiating a drug with known anti-inflammatory properties will be avoided. If this has to be done because of the clinical condition of the patient, study drug administration will be discontinued, and replacement of the patient may be considered. Concomitant medications that are to be expected includeing iron therapy on a regular basis to maintain Tsats>25% and/or ferritin >200, phosphate binders, statins, medication to treat hyperparathyroidism, antihypertensive agents and other drugs to treat known complications of renal failure.

In general, concomitant medication resulting in non-eligibility includes all medication that in the opinion of the investigator would complicate or compromise the study or interfere with the study objectives.

4.9 Return/Recall or Destruction of IMP

Unused drugs will be collected from patients. Accountability by pill counting will be performed by the reseach team and drugs then returned to pharmacy for destruction.

The IMP is provided by Mawdsley who have a standard recall procedure.

5 Study Procedures

5.1 Informed Consent Procedures

Patient information sheet and consent form are separate to this protocol.

A member of the clinical team looking after the patient will approach the potential subject. This will usually be done whilst they are undergoing their regular dialysis or waiting for their dialysis. Patients attend x3/wk for their dialysis, so we would expect them to consider the proposal and if they agree to sign the informed consent (this may be performed either by the clinical team member or a member of the research team). Consent will be countersigned by PI or Local Collaborator delegated by the PI on the delegation log prior to any treatment being given. And that any Nurse undertaking this role will be trained to do so and evidence of this will be in the TMF

Patients attend hemodialysis x3/week indefinitely so it is possible for patients to take days or even weeks to decide.

Use of independent translators will be permitted.

Patients generally attend hemodialysis x3/week until they die, get transplanted or move away (but the last of these is relatively rare). We therefore believe there is opportunity and it will be easy for the research team to feedback information to patients.

5.1 Screening Procedures

A member of the clinical care team will use the electronic Renal Database to identify potential subjects.

The screening/identification will be performed by a member of staff who has clinical responsibilities for renal patients, particularly hemodialysis patients

5.2 Randomisation Procedures (if applicable)

Allocation ratio and any stratification factors (with levels): 1:1 randomisation stratified for Diabetes status and and ESA requirement (adjusted for Hb).

Generation of randomisation codes (manual or automated process): Patients will be allocated to study drug according to recruitment sequence. The drugs sequence will be been randomized by the IMP supplier. Method of randomisation by IMP supplier will be described in a separate randomisation procedure document.

Individual responsible for randomisation and documentation: Study CI or delegated research person will record the recruitment number during the consent procedure.

Access to code break in an emergency: Code Break envelopes will be kept in a locked Office , that can be accessed after contacting nominated Research Persons.

5.3 Schedule of Treatment for each visit

IMP: Oral Pentoxiphylline 400mg once a day

Placebo: Equivalent capsule once a day

5.4 Schedule of Assessment

Subjects will attend their Dialysis Unit (run by BHT)for their standard hemodialysis on Day 1 (visit 1).Subjects will be entered in a run-in period for 2 weeks where biochemistry and haematology will be measure weekly (+/-3 days) to establish baseline. All blood tests will be taken prior to a dialysis session and preferably after an inter-dialytic gap of 48hrs. If the ESA dose changes in the run-in period, the patients will be withdrawn.

Patients will be randomized if the subject meets the inclusion criteria: Subjects will return to the dialysis unit,according to their normal dialysis schedule.During weeks 3-29,subjects will be treated with either IMP or placebo. These will be provided to the patients during the monthly visits. Subjects will be asked to return unused medication for compliance check.

Haematology and Chemistry will be checked every month (+/- 7 days) – see schedule of blood tests. All blood tests will be taken prior to a dialysis session and preferably after an inter-dialytic gap of 48hrs. These tests will include: Hb, Hct, CRP. These monthly blood tests will be taken as part of the patients' standard care and performed in the same local lab as the "additional" "Run-in" (weekly) or "Off-Treatment" (fortnightly) blood tests (that are restricted to Hb, Hct and CRP).

After week 29, treatment (IMP or placebo) will be stopped. Subjects will be asked to return any remaining unused medication. Haematology and Chemistry will be checked every fortnight (+/- 3 days) – see schedule of blood tests. All blood tests will be taken prior to a dialysis session and preferably after an inter-dialytic gap of 48hrs. This "Off-Treatment Follow-up Period" will last for 1 month.

5.4.1 Screening

The investigator or study physician will thoroughly explain the nature and purpose of the study to each subject, as well as the associated procedures and any expected effects and adverse reactions before any study-specific screening procedures are conducted. The subject will be provided with a Subject Information Sheet and given sufficient time and opportunity to inquire about the details of the study and to decide whether to participate. If he or she wishes to participate in the study, the subject will be asked to sign and date an Informed Consent Form (ICF). Study eligibility will be based on investigation of the subject status, after inspection of the following parameters:

- Check of inclusion and exclusion criteria (as described in this protocol);
- Demographics, including gender, race;
- Complete medical history from 30 days before screening;
- Hematology, coagulation, clinical chemistry of most recent clinical visit will be recorded;
- Plasma CRP level.
- ESA dose

Selected subjects who proved to be eligible candidates after investigation of subject status will be enrolled in the study.

5.4.2 Study scheme

Procedure Week:	Run-in: weeks (weekly visits)		Treatment Period (monthly visits)	Non-Treatment Period (Fortnightly visits including end of study visit)
	-2	-1	1 - 26	26-30
24hr Urine collection		x		x
Hematology, Clinical Chemistry [1]	X	X		X
Blood to be taken for storage if separate consent obtained	X	X	X	X
Drug Administration			X	
Vital signs [2]	X	X	X	X
Adverse Events [3]			←----------→	
Concomitant medication [3]			←----------→	

[1]Hematology and clinical chemistry assessments before dialysis.
[2]Including pulse rate and supine BP pre-dialysis.
[3]Adverse events and concomitant medication (including the dose of ESA) will be collected from signing the ICF at screening until last visit.

Consent will be obtained prior to the start of Visit 1 dialysis.

Visit 1 (week -2)
- Arrival at Barts Health Trust (time of day dependent on hemodialysis scheme)
- Vital signs
- Signs and symptoms recording (pre-dialysis, pre-study medication);
- All blood tests will be taken prior to a dialysis session and after an inter-dialytic gap of 48hrs.
- Hematology, clinical chemistry and hs-CRP;
- Haemodialysis;

Visit 2 (on week -1)
- Arrival at BHT (time of day dependent on hemodialysis scheme)
- Vital signs;
- Signs and symptoms recording (pre-dialysis, pre-study medication);
- All blood tests will be taken prior to a dialysis session and after an inter-dialytic gap of 48hrs.
- Hematology, clinical chemistry and hs-CRP, serum for -70C and DNA;
- Haemodialysis;
- Give bottle for patient to collect 24hr urine output (to be collected at next dialysis)

RANDOMISATION will be performed prior to visit 3

Visits 3-8; monthly i.e. weeks 1-26
- Arrival at BHT (time of day dependent on hemodialysis scheme)
- Vital signs;
- Signs and symptoms recording (pre-dialysis, medication);
- All blood tests will be taken prior to a dialysis session and after an inter-dialytic gap of 48hrs.
- Hematology, clinical chemistry and hs-CRP, serum for -70C and DNA(monthly)
- Haemodialysis;
- Collect unused medication and give IMP/placebo for another month.
- Additional visits may be made by the investigators particularly during the first month to ensure patient compliance and to record any AR. These visits will be recorded in the CRF by the most

recent visit number followed by a letter suffix (e.g. visit 3-A or 3-B).

Visit 9

- Arrival at BHT (time of day dependent on hemodialysis scheme)
- Vital signs;12-lead ECG (pre-dialysis);
- Signs and symptoms recording (pre-dialysis, medication);
- All blood tests will be taken prior to a dialysis session and after an inter-dialytic gap of 48hrs.
- Hematology, clinical chemistry and hs-CRP, serum for -70C and DNA(monthly)
- Hemodialysis;
- Give bottle for patient to collect 24hr urine output (to be collected at next dialysis)

Visit 10 - 11; fortnightly i.e. weeks 28 - 30

- Arrival at BHT (time of day dependent on hemodialysis scheme)
- Adverse Event recording including any medication changes;
- All blood tests will be taken prior to a dialysis session and after an inter-dialytic gap of 48hrs.
- Hematology, clinical chemistry and hs-CRP, serum for -70C and DNA(monthly)
- Hemodialysis;

Protocol for Intravenous Iron Dosing (Venofer, iv) – Local Protocol

Ferritin	**Dose of iv Fe**	**Frequency**
<100	100mg	Twice a week
100-200	100mg	Once a week
200-500	100mg	Once every 2 weeks
>500		nil

Protocol for ESA Dosing (Local Protocol):

Target Hb range will be 10 -12g/dl. ESA dose will be increased or decreased by 25-50% depending on Hb (2 consecutive readings)

Hb (g/dl)	**Dose change of ESA**
>15	Stop ESA and venesect 1 unit blood or discard 1 HD circuit aft HD
14-15	Nil – ESA stopped
13-14	Cut 50%
12-13	Cut 25%
9 – 10	Increase 25%
<9	Increase 50%

5.5 Follow up Procedures

Haematology and Chemistry will be checked every fortnight (+/- 3 days) – see schedule of blood tests. All blood tests will be taken prior to a dialysis session and preferably after an inter-dialytic gap of 48hrs. This "Off-Treatment Follow-up Period" will last for 1 month.

5.6 Laboratory Assessments

"Standard Lab" Tests for the study will be: Hb, Hct and CRP.
Cytokine profiles and DNA telomere lengths will also be measured.
However, we note that the standard care of HD patients at Barts Health Trust undergo regular monthly blood tests that include these and more extensive "LFT" and "Bone Profiles". These results will be available to the researchers.

Parameter	Vacutainer	Volume per sample (mL)	Number of samples	Total volume (mL)
Clinical biochemistry	1x serum	7	12	84
Blood to be taken if separate consent given permitting storage of blood	1xserum	7	12	84
	1x EDTA	3.5	12	42
Haematology	1x EDTA	3.5	12	42
			TOTAL VOLUME	**252**

5.7 Radiology Assessments

Cardiovascular imaging will be performed at baseline and at 6 months. The effect IMP has on the following cardiovascular parameters will be examined:

- mean target-to-background ratio across a substantial portion of artery (typically aorta, supraaortic vessels and femoral arteries) using 18-FDG-PET (time-of-flight). This will allow us to assess whether IMP has an anti-inflamatory effect on the blood vessels
- vascular stiffness measures (e.g. aortic distensibility at three levels of aorta (mmHg-1), pulse wave velocity (m/s) and carotid distensibility (mmHg-1))using magnetic resonance (MR) imaging.

For further details, refer to Appendix 1 and 2

5.8 End of Study Definition

This will be considered to be after the last visit of the last participant and 6 months after analysis of end-point evaluations.

5.9 Procedures for unblinding (if applicable)

Emergency Unblinding Procedures including Out of Hours:

- Uncoding envelopes will be kept in the Renal Office (locked). Permission to access will be given by: Prof M Yaqoob or Dr S Fan or Research Clinic Fellow (to be appointed). Contact details will be with switchboard at Barts Health NHS Trust.

- After a patient is unblinded, the patient will be given an option to undergo the end-point tests and the 1 month "off drug" follow up.

Unblinding should only occur in the case of a medical emergency.

5.10 Subject Withdrawal

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The responsible investigator can also withdraw a subject if continuing participation is in his opinion deleterious for the subject's well being. Subjects can also be withdrawn in case of protocol violations and non-compliance (as deemed significant by the investigators).

5.11 Data Collection and Follow up for Withdrawn Subjects

In case of withdrawal because of severe or serious adverse event haematological, blood chemistry and urine laboratory tests or other special examinations may be performed.

Dropouts will not be re-included in the study. Their inclusion and treatment numbers must not be re-used. Dropouts due to withdrawal of informed consent or non-study drug related events might be replaced in order to obtain a sufficient number of subjects completing the study. The possible need for replacement of dropouts will be discussed by the primary investigator and responsible medical officer of the Sponsor.

When a subject withdraws from the study, the subjects will be asked if they are willing to continue in the 1 month "non-treatment" study period. When a subject withdraws, the second radiological assessment may be conducted with patient consent. However, the patients are made aware in the PIS that audits of patients' blood results are continuously performed as part of standard NHS care within the Renal Unit at Barts Health Trust and this will continue.

All adverse events and concomitant medication will be entered in the CRF.

6 Laboratories (if applicable)

6.1 Local Laboratories

Laboratory for the Biochemistry and Haematology tests will be the Pathology Department at Barts Health NHS Trust.

6.2 Sample Collection/Labelling/Logging

The laboratory will be blinded to the study drug allocation. Patient samples will not be anonymised or coded. The results of study biochemistry and haematology tests will be available to clinicians looking after the patients for clinical governance reasons.

Blood (if separate consent if given) will be stored at -70C (with a target of 60mins between phlebotomy and storing at -70C).
Standard biochemical and haematological samples will be kept at room temp and delivered through the hospital portering system.

All blood tests will be taken prior to a dialysis session and preferably after an inter-dialytic gap of 48hrs.

7 Pharmacovigilance

7.1 General Definitions

7.1.1 Adverse Event (AE)/ Adverse Reaction (AR) / Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational drug. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded on the adverse event data collection form. The intensity of these adverse events will thereby be graded by the investigator on a three-point scale as defined below:

Mild	discomfort noticed but no disruption of normal daily activity
Moderate	discomfort sufficient to reduce or affect normal daily activity
Severe	inability to work or perform daily activity

The chronicity of the event will be classified by the investigator on a three-item scale as defined below:

Single occasion	single event with limited duration
Intermittent	several episodes of an event, each of limited duration
Persistent	event which remained indefinitely

For each adverse event the relationship to drug (definite, probable, possible, unknown, definitely not) as judged by the investigator as well as eventual actions taken will be recorded. The occurrence of an adverse experience that is fatal, life-threatening, disabling or requires or prolongs in-patient hospitalisation or causes congenital anomaly will be described according to guidelines as "serious" adverse. Important medical events that may not be immediately life threatening or result in death or hospitalisation may be considered a serious adverse event when, based on appropriate medical judgement, they may jeopardise the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.

7.2 Notification and reporting Adverse Events or Reactions

If the AE is not defined as SERIOUS, the AE is recorded in the study file and the participant is followed up by the research team. The AE is documented in the participants' medical notes and the CRF.

7.3 Notification and Reporting of Serious Adverse Events/SUSAR

7.3.1 Serious Adverse Event (SAEs)

All SAEs will be recorded in the subjects' medical notes, the CRF, the sponsor SAE form and reported to the Joint Research Management Office (JRMO) within 24 hours of the CI or PI or co-investigators becoming aware of the event. Nominated co-investigators will be authorised to sign the SAE forms in the absence of the CI at the co-ordinating site or the PI at the participating sites.

The following SAEs do not have to be reported but will be captured by the investigators:

- Any routine surgery/procedure that is related to dialysis access (Peritoneal dialysis and haemodialyis, e.g. for malpositioned, non-functioning PD catheters or for creation of AV-graft fistulae).
- Any surgery/procedure that had been planned prior to trial enrollment.
- Any admissions for renal transplantations
- Any admission that are known to be related to HD such as infections, inadequate dialysis, problems with access, hypotension, fluid overload.
- Any admission for related to missed dialysis sessions.

7.3.2 Suspected Unexpected Serious Adverse Reactions (SUSARs)

Will be reported to the JRMO/ main REC within 48 hours of the CI or co-investigator becoming aware of the event. SUSARs should be reported to the sponsor (JRO Office) within 24 hours as the sponsor has a legal obligation to report this to the MHRA within 7 days (for fatal or life-threatening SUSARs) or 15 days for all other SUSARs. The CI will need to complete the SUSAR form in conjunction with the sponsor SAE form to be sent to the MHRA by the sponsor.

The original and any subsequent follow up of Serious Adverse Event Forms and SUSUAR forms (where applicable), together with the sending confirmation sheet must be kept with the TMF at the study site.

7.4 Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical trial subjects from any immediate hazard to their health and safety, in accordance with Regulation 30. The measures should be taken immediately. In this instance, the approval of the Licensing Authority Approval prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor, Main Research Ethics Committee (via telephone) and the MHRA (via telephone for discussion with the medical assessor at the clinical trials unit) of this event **immediately**.

The CI has an obligation to inform both the Sponsor,MHRA and Main Ethics Committee **in writing within 3 days**, in the form of a substantial amendment. The sponsor (JRMO) must be sent a copy of the correspondence with regards to this matter.

7.5 Annual Safety Reporting

The Development Safety Update Reports (DSUR) will be sent by the CI to the sponsor, the MREC and MHRA (the date of the anniversary is the date on the "notice of acceptance letter" from the MHRA) using the Sponsors DSUR template form. The CI will carry out a risk benefit analysis of the IMPs encompassing all events having arisen on the trial.

The CI will send the Annual Progress Report to the main REC using the NRES template (the anniversary date is the date on the MREC "favourable opinion" letter from the MREC) and to the sponsor.

Procedures for reporting blinded SUSARs

Treatment code for the patient is broken in the reporting of a SUSAR. However, the blind should be maintained, where possible and appropriate, for staff that are involved in data analysis and interpretation.

It is recommended that in the case of a blinded study, the case is assessed for seriousness, expectedness and causal relationship as if it was the tested IMP that caused the reaction. If the case appears to be a SUSAR then it should be unblinded .

If the administered product is the tested IMP, the case would be reported as a SUSAR to the MHRA/ appropriate Main Research Ethics Committee.

If the administered product is a comparator with a marketing authorisation, the adverse reaction should be reassessed for expectedness according to the study protocol. If the adverse reaction is unexpected then the SUSAR should be reported; otherwise it is an expected serious adverse reaction which still requires reporting to the sponsor within 24 hours.

7.6 Overview of the Safety Reporting Process/Pharmacoviligance responsibilities

The CI has the overall pharmacovigilance oversight responsibility. The CI has a duty to ensure that pharmacovigilance monitoring and reporting is conducted in accordance with the sponsor's requirements.

7.7 Pregnancy

If a patient becomes pregnant whilst involved in this CTIMP, it is not considered to be an SAE or an AE. However, it is an event that requires monitoring and follow up. If a patient, or his partner, becomes pregnant whilst enrolled in a CTIMP in which the foetus has been exposed to an investigational medicinal product, immediate reporting to the sponsor is required (within one working day of the PI/CI becoming aware of the event) using a JRMO pregnancy template form. The CI/PI has the responsibility to ensure that the pregnancy form is completed and sent to the sponsor within the agreed timelines. Please state whether the patient can continue on the study or whether the patient has to be prematurely withdrawn from the study here.

The PI/CI also must follow up the pregnancy until delivery as well as monitoring the development of the newborn for the appropriate time (1 month) after birth. Any

events that occur during this time that could be considered to be a SAE must be reported to the sponsor in line with section above, utilising the sponsor SAE reporting form.

8 Statistical Considerations

Missing and spurious data

Data will undergo review to identify missing and spurious data. The data review will be an integral part of the report and includes the decisions on and documentation of such data.

Only subjects with a baseline sample and at least one sample obtained during the treatment phase will be included in the analysis. Subjects who have discontinued at any time will undergo safety evaluations; reasons for discontinuation will be evaluated. Statistical plan and methods to be employed

The statistics of this study entails a "per protocol" analysis and an Intention to Treat analysis where the last follow-up data point is carried over.

Selection of subjects to be included in the analysis

For intention to treat analysis, all data that meaningfully contribute to the objectives of the study will be included. The data of all subjects will be included in the safety analysis.

8.1 Primary Endpoint Efficacy Analysis

We shall be compare δEPO/kg requirement between the 2 groups using unpaired student t-test. We expect that to compare the means of the run-in and last 2 visits of each patient will be used for primary end-point analyses (ITT), and the last 2 visits of each patient when taking IMP or placebo (Per Protocol).

8.2 Sample Size

From internal audit data on ESA usage, we assume the average dose of ESA will be 12000iu/week (with SD=500)

If the placebo group have Hb=10g/dl, the ESA/Hb ratio = 1200

The study by Cooper et al showed that 4 months treatment increased Hb levels by 25%.

We wish to power this study to demonstrate that IMP will increase ESA response by 5%

If we assumed the SD for ESA/Hb = 100, this will mean that for an alpha level of 5% (95%CI) and a beta level of 5% (statistical power of 95%) we shall need 30 patients in each group. We assume that there will be a 50% drop out (transplantation and patient withdrawal) so we plan to recruit 100 patients

9 Data Handling & Record Keeping

9.1 Confidentiality

The Chief Investigator has a responsibility to ensure that patient confidentiality is protected and maintained. They must also ensure that their identities are protected from any unauthorised parties. Information with regards to study patients will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval.

The Investigator as well as the study team must adhere to these parameters to ensure that the Patient's identity is protected at every stage of their participation within the study. To ensure this is done accordingly, each patient, at time of consent must be allocated an unique screening number by either the PI or a member of the study team before undergoing any screening procedures.The patients initials (the first letter of their first name and the first letter of their last name) should be used as a means of de-identifying parameters. This information should be kept on a screening log, which should be updated accordingly throughout the study. Once the patient has completed screening procedures and is enrolled onto the study, the patient will be allocated a randomisation number and entered on an enrolment log.

If any patient information needs to be sent to a third party (including correspondence/communication to central laboratories, CROs, sponsor) the PI and the study team should adhere to patient de-identified parameters. This includes the patient initials, date of birth, gender as well as the unique study ID/randomisation number. Any information that is to be collected by these third parties will utilise these coded details for any revelant documents as well as maintaining databases.

- What identifiable information will be collected from the subjects? Nil expected.
- Who will have access to the Information and why? Only researchers or those required for audit of study
- The Chief Investigator is the 'Custodian' of the data.
- Patient identifiable details will not be transferred outside the EU.
- The patients will be anonymised with regards to any future publications relating to this study.

9.2 Case Report Form

CRF Data Collection Summary					
	Screening	Consent	Run-in	Treatment	Off-Treatment FU
Informed Consent		x			
Inclusion/Exclusion Criteria check	x	x			
Medical History Recorded (in full)	x				

Vital Signs (Blood Pressure)	x		x	x	x
PET-CT			x		x
Cardiac MR			x		x
Weight	x		x	x	
ESA dose	x		x	x	X
Current Medical Conditions			x	x	x
Adverse Event			x	x	x
Concomitant Medications	x		x	x	x
Local Lab assessment	x		x	x	x
Demographics (including Date of Birth and Gender)	x				

9.3 Record Retention and Archiving

During the course of research, all records are the responsibility of the Chief Investigator and must be kept in secure conditions. When the research trial is complete, it is a requirement of the Research Governance Framework and Trust Policy that the records are kept for a further 20 years. For trials involving BH Trust patients, undertaken by Trust staff, or sponsored by BHT,, the approved repository for long-term storage of local records is the Trust Modern Records Centre which is based at 9 Prescot Street.

9.4 Compliance

The CI will ensure that the trial is conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, and all subsequent amendments, Trust and Research Office policies and procedures and any subsequent amendments.

9.5 Clinical Governance Issues

9.5.1 Ethical Considerations

This protocol and any subsequent amendments, along with any accompanying material provided to the patient in addition to any advertising material will be submitted by the Investigator to an Independent Research Ethics Committee. Written Approval from the Committee must be obtained and subsequently submitted to the JRMO to obtain Final R&D approval, prior to any activity taking place or changes implemented.

9.6 Quality Control and Quality Assurance

9.6.1 Summary Monitoring Plan

Refer to PEAR Monitoring Plan for further details

The first monitoring visit is due within three months of the first patient being enrolled at a site. Thereafter monitoring visits will occur every four months. The monitoring will be a combination of completion of self monitoring forms and a yearly onsite visit.

Source data verification (SDV) will be performed, as detailed in the monitoring plan. This will involve direct access to patient notes and include the review of consent forms and other relevant investigational reports. Missing data will be sought, unless confirmed as not available.

Non-commercial central laboratories will be monitored though on site and self monitoring during their participating in the trial, as detailed in the monitoring plan.

9.6.2 Audit and Inspection

This study may be audited by representatives from the sponsor or IMP supplier. The investigator will be informed of any audit outcome. Investigators are obliged to cooperate in any audit, allowing the auditor direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor to discuss any findings or issues. Audit may occur at any time during or after completion of the study.

Inspections may be carried out by the Competent Authority at any time and the investigator should notify the sponsor immediately if there are any such plans for an inspection.

9.7 Serious Breaches in GCP or the Trial Protocol

The sponsor of the Clinical Trial is responsible for notifying the licensing authority in writing of any serious breach of:

The conditions and principles of GCP in connection with that trial; or
The protocol relating to the trial, as amended from time to time in accordance with regulations 22 to 25, within 7 days of becoming aware of that breach.

For the purposes of this regulation, a 'serious breach', is a breach which is likely to effect to a significant degree:
The safety or physical or mental integrity of the subjects of the trials; or
The scientific value of the trial.

The CI is responsible for reporting any serious breaches to the sponsor (JRMO) **within 24 hours**. The sponsor will notify and report to the MHRA within 7 working days of becoming aware of the serious breach.

9.8 Non-Compliance

Is defined as a noted systematic lack of both the CI and the study staff adhering to SOPs/protocol/ICH-GCP and UK regulations, which leads to prolonged collection of deviations, breaches or suspected fraud.

These non-compliances may be captured from a variety of different sources including monitoring visits, CRFs, communications and updates. The sponsor will maintain a log of the non-compliances to ascertain if there are any trends developing which to be

escalated. The sponsor will assess the non-compliances and action a timeframe in which they need to be dealt with. Each action will be given a different timeframe dependant on the severity. If the actions are not dealt with accordingly, the JRMO will agree an appropriate action, including an on-site audit.

10 Trial Committees

The chair the Data Safety Committee will not be an active member of PEAR. The committee will review the progress of the study and issues arising from the study. This will include review of SAE to determine if there are any trends that would suggest concerns about the conduct of the study.

A separate Renal Research committee meets regularly throughout the year. Concerns of CI or study members about this study can be discussed at this forum to provide external expertise.

11 Publication Policy

Only anonymised data will be used for publication. We shall attempt to publish results in peer reviewed journals.

Appendix 1: PET Imaging Protocol

Abbreviations

CECM	Centre for Experimental Cancer Medicine
CT	Computed tomography
FDG	Fluorodeoxyglucose
IMP	Investigational Medicinal Product
MDT	Multi Disciplinary Team
PET	Positron Emission Tomography
SOP	Standard Operating Procedures

Contacts

Study Team

Chief Investigator

Professor Magdi Yaqoob
St. Bartholomew's Hospital
Barts Health NHS Trust
London, EC1 7BE

PET

PET Experts

Dr Jan Hikman
St. Bartholomew's Hospital
Barts Health NHS Trust
West Smithfield
London, EC1 7BE

Tel: 020 3465 5922
Email: Jan.Hikmanl@bartsandthelondon.nhs.uk

PET Physicist

Dr. Yassine Bouchareb
St. Bartholomew's Hospital
Barts Health NHS Trust
West Smithfield
London, EC1 7BE
020 3465 6902
Email:yassine.bouchareb@bartshealth.nhs.uk

PET Technologists

Craig Copland
St. Bartholomew's Hospital
Barts Health NHS Trust
West Smithfield
London, EC1 7BE
020 3465 5883
Email: craig.copland@bartshealth.nhs.uk

Rob Punjani
St. Bartholomew's Hospital
Barts Health NHS Trust
West Smithfield
London, EC1 7BE
020 3465 5883
Email: rob.punjani@bartshealth.nhs.uk

Introduction

This manual summarises the agreed PET protocol for assessing the whole body distribution of arterial inflammation in renal failure patients. The patients in this study will have confirmed renal failure and will be on dialysis.

1.1. PET/CT

All PET acquisitions should be accompanied by a low-dose CT procedure to allow for attenuation correction and co-localisation of PET data. The parameters of these CT acquisitions should be such that the estimated effective dose is no more than 5 mSv each.

1.2. FDG-PET/CT

As plasma clearance in these patients is delayed, dynamic imaging from 0 to 60 minutes over the area starting from the Arch of the Aorta (upper limit: arch of the Aorta and lower limit: the axial extent of PET Field-of-View) will be performed followed by whole body imaging at 90minutes'

1.2.1. Glucose Measurement

Patients undergoing FDG-PET/CT should have a blood glucose measurement carried out prior to administration of ^{18}F-FDG. FDG administration should only proceed if blood glucose is < 10.0 mmol/l.

1.2.2. Dose

The administered activity of ^{18}F-FDG should be < 200 MBq due to renal failure in these patients. 200MBq must not be exceeded.

1.2.3. Type of scan

Helical CT acquisition for the low-dose CT from the base of the skull to the knee joints.
Whole Body PET data: Multiple bed positions should be acquired from the knee joints to the base of the skull. The time per bed position should be 2 minutes and the PET data acquisition should not exceed a total of 30 minutes.

1.2.4. Uptake period

Dynamic scan start at injection time (20 seconds delay); followed by whole body scan at 90 minutes from injection time.

1.2.5. Total Scan time

No more than 1 x 60 minutes and 1 x 35 minutes (max. 30 min PET emission).
Blood sampling: 3 samples (3mls each) at minutes 1, 2 and 3 form the injection of activity; then 3 samples (3mls each) at minutes 5, 7 and 9 followed by 5 samples at 19, 29, 39, 49 and 59 minutes) (total 11 samples =33 mls blood smaple per scan)

1.2.6. FDG-PET Local Analysis

Image analysis will be performed on a dedicated workstation. Using the CT images, the vasculature will be divided into carotid arteries, the aorta, iliac and femoral arteries. The common and external iliac arteries will be combined and treated together as ''iliac artery''; similarly, the common femoral and superficial femoral arteries are amalgamated into the single label of ''femoral artery.'' The transition point between iliac and femoral arteries is the inguinal ligament.

Arterial 18F-FDG uptake (as a measure of arterial inflammation) in the legs and neck are measured by drawing a region of interest (ROI) around the artery on every slice of the coregistered transaxial PET/CT images. On each image slice, the mean and maximum standardized uptake values (SUVs) of 18F-FDG in the ROI (containing the arterial wall and the lumen) are calculated as the mean and maximum pixel activity. The SUV is the decay-corrected tissue concentration of 18F-FDG (in kBq/g), adjusted for injected 18F-FDG dose and body weight (in kBq/g), and is a well-recognized method for quantification of 18F-FDG PET data.
By averaging SUVs for all artery slices within an arterial territory, mean and maximum SUVs can be derived for each region. These SUVs are normalized to blood 18F-FDG activity by division by an average blood ROI (at least 8 venous ROI measurements), estimated from either the inferior vena cava (leg studies) or the jugular vein (carotid studies). This calculation results in an arterial TBR measure, which is reported subsequently.

12 APPENDIX 2: Image Acquisition Form

ACQUISITION DATA

Type of Scan:	FDG-PET/CT
Scan acquired at: *(Name of department / PET Centre)*	
Patient initials:	
Patient date of birth:	
Patient trial number:	
Referring Consultant / Study Name or Code:	
Consultant telephone number:	
Consultant fax number:	
Hospital Address:	
Date of scan:	

15.2 Protocol: PEAR Study- Experimental outcomes

Full Title	PEAR study: experimental outcomes version
Sponsor	Queen Mary, University of London *Contact person of the above sponsor organisations is:* Head of Research Resources Joint Research Management Office 5 Walden Street London E1 2EF Phone: 020 7882 7260 Email: sponsorsrep@bartshealth.nhs.uk
REC Reference	
Chief Investigator	*Prof. M.M. Yaqoob*

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18.

2. GLOSSARY of Terms and Abbreviations

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
JRMO	Joint Research Management Office
NHS REC	National Health Service Research Ethics Committee
NHS R&D	National Health Service Research & Development
Participant	An individual who takes part in a clinical trial
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

3. SIGNATURE PAGE

Chief Investigator Agreement

The clinical study as detailed within this research protocol **(Version 1.0, dated 14.Nov.2014)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Chief Investigator Name: Prof. M.M. Yaqoob

Chief Investigator Site: Royal London Hospital

Signature and Date:

Principal Investigator Agreement *(if different from Chief investigator)*

The clinical study as detailed within this research protocol **(Version 1.0, dated 14.Nov.2014)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Principal Investigator Name: Dr. S. Fan

Principal Investigator Site: Royal London Hospital

Signature and Date:

4. SUMMARY/SYNOPSIS

Short Title	PEAR study : experimental outcomes
Methodology	Laboratory analysis of blood samples stored for future research as a part of PEAR study
Research Sites	Barts Health NHS Trust- Royal London Hospital
Objectives/Aims	To study the effect of Pentoxifylline on cytokine levels, leucocyte DNA telomere length shortening and Highly sensitive c reactive protein compared to control group.
Number of Participants/Patients	69
Main Inclusion Criteria	Not applicable
Statistical Methodology and Analysis (if applicable)	Analysis will include descriptive statistics, t-test, Paired-t variance and covariance and use of non-parametric statistics when required.
Proposed Start Date	15.Dec.2014
Proposed End Date	15. Dec.2015
Study Duration	Not applicable

5. INTRODUCTION

Anaemia is a common complication of End Stage Renal Disease (ESRD). This usually happens as a result of reduced production of erythropoietin hormone by the kidneys. Anaemia as a result of ESRD is treated by giving injections of erythropoiesis stimulating agents (ESAs) which have an action similar to natural erythropoietin.

Some patients with ESRD are less responsive or resistant to the action of ESAs consequently requiring higher doses of ESAs. High ESA requirement is a surrogate marker of increased all cause cardiovascular mortality & morbidity in ESRD patients. Elevated levels of inflammatory cytokines as chronic inflammation is one of the major factors associated with ESA hypo-responsiveness. DNA telomere length shortening is another surrogate marker for cardiovascular risks in haemodialysis patients which is associated with chronic inflammation.

There is emerging evidence for Pentoxifylline, a non-selective phosphodiesterase inhibitor as a potential treatment for ESA hypo responsiveness. Pentoxifylline (used for twenty years in the treatment of peripheral and cerebral vascular diseases), has potent haemorheological and anti-inflammatory properties (secondary to inhibition of phosphodiesterases). Pentoxifylline is associated with reduction of pro inflammatory cytokines such as tumour necrosis factor (TNF)-α, Interferon (IFN)- γ and Interleukin (IL)- 6 in haemodialysis and CKD patients as well as improving haemoglobin.

Currently a Single Centre randomized placebo controlled, double blinded study to see if Pentoxifylline can help End Stage Renal Disease Patients with Erythropoietin Resistance (PEAR study) is underway at Barts Health NHS Trust (Rec number: 12/LO/1635 EudraCT Number: 2011/006168/30).

As a part of this research, some patients recruited in the study have consented for additional blood tests to be taken during the course of the study for future research. Therefore we intend to investigate effect of pentoxifylline on cytokine levels, inflammation and consequently on DNA telomere length shortening in our patient group.

6. TRIAL OBJECTIVES

Primary Endpoint

To study the effect of pentoxifylline on cytokine levels in ESRD patients on haemodialysis with hypo-responsiveness to erythropoiesis stimulating agents or ESAs.

Secondary Endpoint

To study effect of pentoxifylline on leucocyte DNA telomere length shortening and highly sensitive CRP levels

7. METHODOLOGY

Inclusion Criteria

Not applicable for the proposed study as we are aiming to analyse stored samples collected from consenting patients during PEAR study.

Exclusion Criteria

Not applicable for the proposed study as we are aiming to analyse stored samples collected from consenting patients during PEAR study.

Study Design / Plan

Pseudo-anonymised samples for future research are stored in Human Tissue Authority (HTA) approved lab will be analysed for cytokine levels, highly sensitive c reactive protein and leukocyte DNA telomere length shortening. Pseudo-anonymised samples will be unblinded at the end of relevant laboratory investigations. Unblinding of lab samples will be done after unblinding for PEAR study done. However samples shall remain pseudo-anonymised. Samples will be analysed in batches.

8. STUDY PROCEDURES

Blood samples are only taken from the participants of PEAR study who have consented for samples to be stored for future research. Samples are pseudo-anonymised before storage. Samples will be analysed in batches. Results and relevant pseudo-anonymised data will be stored in secured trust computers. Available results will be correlated with intervention in PEAR study once PEAR study data is unblinded.

9. STATISTICAL CONSIDERATIONS

This is a pilot study generating hypothesis. Sample size considerations do not apply as this project involves analysing samples stored for future research as a part of PEAR Study. Analysis will include descriptive statistics, t test, Paired t variance and covariance and use of non-parametric statistics when required.

10. ETHICS

This study does not raise any significant ethical issues. This study falls into criteria of studies which do not require any formal ethics review as per the National Research Ethics (NRES) guidelines.

Patients have already consented for blood tests to be done and stored for future research. Anonymity shall be maintained (pseudo-anonymised). Samples shall be stored in HTA approved laboratory.

11. SAFETY CONSIDERATIONS:

There are no safety concerns.

12. DATA HANDLING AND RECORD KEEPING:

- Confidentiality

Information related to participants should be kept confidential and managed in accordance with the Data Protection Act, NHS Caldecott Principles, The Research Governance Framework for Health and Social Care, and the conditions of Research Ethics Committee Approval.

- Record Retention and Archiving

When the research trial is complete, the data will be stored as per research Governance Framework and Trust Policy that the records are kept for a further 20 years.

13. LABORATORIES :

Samples will be processed in Immunology/Serology Laboratory at Royal London Hospital. No additional laboratory preparation is required.

Samples have already been pseudo-anonymised at the time of collection. A detailed log of samples taken to laboratory for analysis will be maintained. Optimal temperature conditions will be maintained during the transit to maintain viability of the samples.

14. PRODUCTS, DEVICES, TECHNIQUES AND TOOLS :

Cytokine analysis

Cytokine assays will be done using commercially supplied kits (R&D Systems) using standard manufacturer's protocol for analysis. Initially the specific anti-cytokine antibody (capture antibody) is bound to a polystyrene microplate. Unbound capture antibody is then washed away. Plates are blocked and washed. Samples or standards are added and any chosen cytokine present is bound by the immobilized antibody. Unbound materials are then washed away. Streptavidin-Horseradish Peroxidase (HRP) is used to bind to the detection antibody. Unbound streptavidin-HRP is washed away. Tetramethylbenzidine (TMB) substrate solution is added to the wells and a blue colour develops in proportion to the amount of analyte present in the sample. Colour development is stopped turning the colour in the wells to yellow. The absorbance of the colour at 450 nm is measured.

DNA telomere length analysis:

Telomere length will be measured by a qPCR assay that compares the mean telomere repeat sequence copy number (T) to a reference single-copy gene copy number (S) in each sample which is then validated by comparison with Southern blot terminal restriction fragment analysis. PCRs to be conducted using a Qiagen Rotor-Gene Q real time PCR cycler (Qiagen, Manchester UK). Telomere length was measured in T/S ratio units. The relative quantity of the single copy gene (S) in each experimental sample was expressed as the level of dilution of the reference DNA sample needed to match it to the experimental sample with regard to the number of cycles of PCR needed to generate a given amount of single copy gene PCR product during the exponential phase of the PCR (T). For each experimental sample the ratio of these dilution factors is the relative telomere to single copy gene (T/S) ratio. Thus T/S = 1 when the unknown DNA is identical to the reference DNA in its ratio of telomere repeat.

15. SAFETY REPORTING

Not applicable as research is done on samples already stored for future research.

16. MONITORING &AUDITING

Will be done as per according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement as directed by JRMO.

17. TRIAL COMMITTEES

This usually consists of the CI, PI and investigator Dr. Tarun Kaushik. Trial Committee will meet at regular intervals to assess progress of the study.

18. FINANCE AND FUNDING

Barts Cardiovascular Biomedical Research Unit (BRU) is funding the project.

19. INDEMNITY

Indemnity will be provided by the sponsor,
Joint Research Management Office RMO,
Queen Mary University of London,
Queen Mary Innovation Centre,
5, Walden Centre
London E1 2EF.

20. DISSEMINATION OF RESEARCH FINDINGS:

Study results disseminated in internal report, conference presentations and peer reviewed scientific journals.

Investigator:

Dr. Tarun Kaushik
Clinical Research Fellow
WIliam Harvey Research Institute
Queen Mary University, London
Charter House square,
London EC1M 6BQ

15.3 Protocol: PEAR Study- patient information sheet and consent form

Barts Health NHS
NHS Trust

Patient Information for Study:

PEntoxifylline in Anaemia Resistant to erythropoietin (PEAR)

Chief Investigator: **Prof M Yaqoob, Barts Health NHS Trust**
Principal Investigator: **Dr Fan**

'You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- *Part 1 tells you the purpose of this study and what will happen to you if you take part.*
- *Part 2 gives you more detailed information about the conduct of the study.*

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1.
What is the purpose of the study?

Pentoxifylline
Pentoxifylline has been widely used for over 20 years to treat people with circulation problems (both of the legs and the brain). The drug is generally very safe.

In addition to its effect on circulation, people have noticed that it also has potent anti-inflammatory properties.

Inflammation is often the reason why people on dialysis are anaemic (have low Hb) despite the use of a hormone called erythropoietin stimulating agent (ESA or otherwise commonly called EPO). A small study showed that this treatment could improve the Hb level of patients who did not originally respond to EPO. Unfortunately the study was small and needs to be repeated before we can be sure it is effective.

Research Study Question
We want to treat 2 groups of patients. One will receive Pentoxifylline and the other will receive "placebo" (a "dummy tablet). We want to compare the Hb level of patients in the 2 groups.

Why have I been chosen?
We have invited you to help us because you have been identified as requiring a high dose of EPO. This means that you are at risk of becoming anaemic or having side-effects from EPO.

Do I have to take part?
'No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive'.

What will happen to me if I take part?
If you agree to take part, we will ask you to sign a consent form.

Sometimes we don't know which way of treating patients is best. To find out, we need to make comparisons between the different treatments. We put people into groups and give each group a different treatment; the results are compared to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). The results are then compared.

We will randomly put you into either the Pentoxifylline or Placebo group. You will have a 50:50 chance of being put into the Pentoxifylline treatment group, but neither you nor your doctor will know (the study is "blinded").

If you are in the study, you will be treated for 6 months. During these 6 months, you will be reviewed regularly by a member of the Research Team. We shall also take 2 extra blood tests every month (2 teaspoonfuls) from your dialysis machine. These blood tests are designed to look at how you respond to your EPO and how much "inflammation" is in your body. It also involves studying your DNA. At the end of the 6 months, you will stop the tablets, but we will still monitor your blood tests every 2 weeks for 2 months.

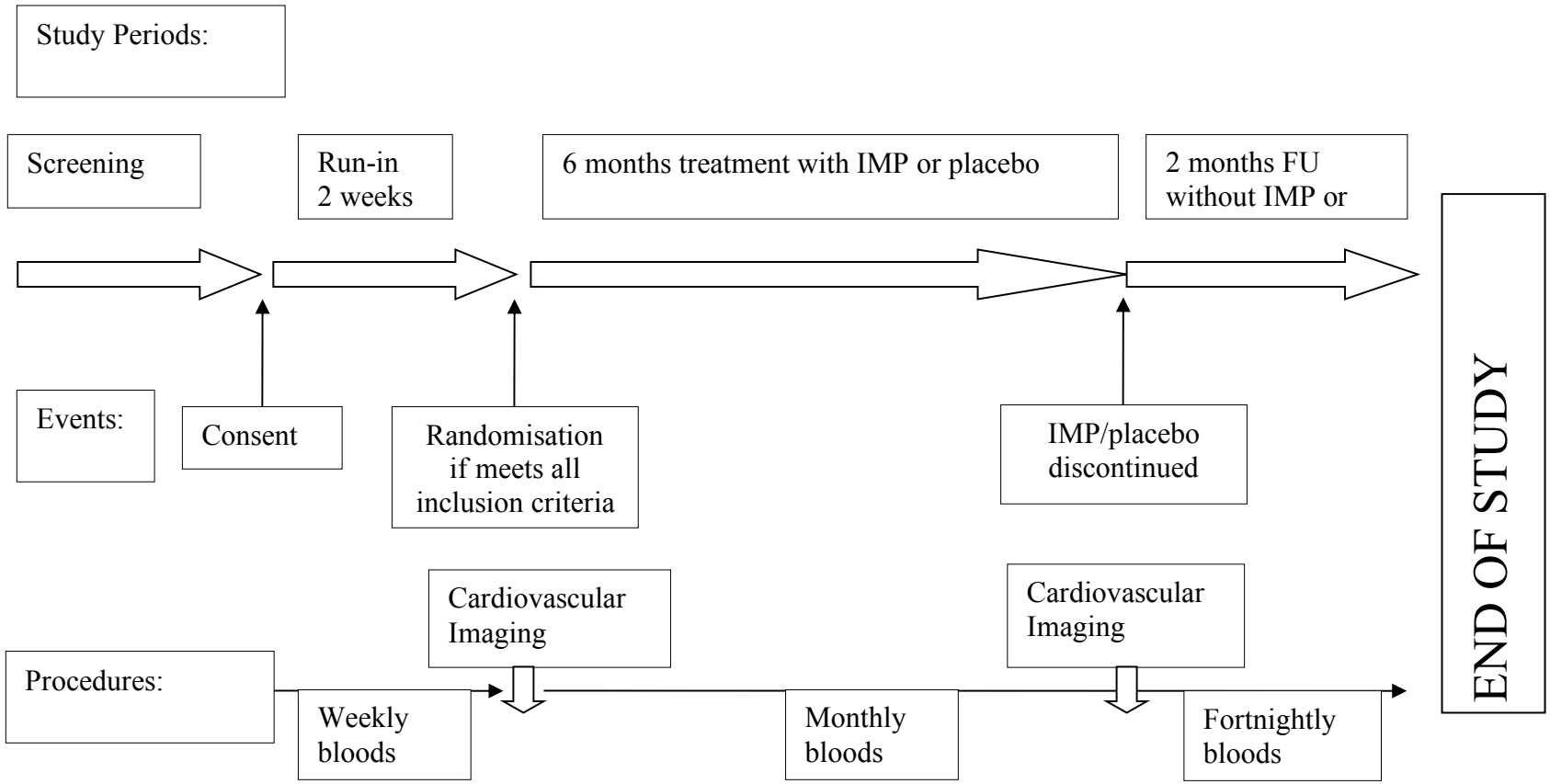
You will also have special "X-Rays" that are designed to look at your heart and blood vessels. These tests will be done over a 2-hour period and you will be asked to attend the hospital. You will have an X-ray test before and at the end of the study. One of the X-ray test is called a "PET-CT". This involves an injection of a radioactive dye. Blood will then be taken at regular intervals over 1 hour (about 60mLs). The level of radioactivity is 6 times the normal background radiation level in the UK. It will not affect your routine daily life. You will also have another special X-Ray called "Magnetic Resonance" (MRI) scan that looks at your heart. If you are afraid of being in closed spaces (claustrophobic) or if you have any metallic implants such as a hip replacement or a pacemaker, you must tell us as you will not be able to have this scan.

Your general renal / dialysis care will not be affected. Your participation in this study will not affect the chances of you getting a kidney transplant.

Expenses and payments:
Most visits will be carried out when you come in for dialysis. There will be two extra visits for the specialized X-ray tests. We shall arrange the transport for you or reimburse any extra travel costs that you incur.

What do I have to do?
If you agree to do this study, you will need to take the tablets we select for you once a day for 6 months. You will have a few extra blood tests (taken when you are on dialysis) and special X-rays that will require 2 extra visits to the hospital. If you have a problem or if you dislike your treatment, you can withdraw from the study at any time. Other aspects of your care will not be affected.
The Timetable for what will happen is shown below:

Summary of Study Plan



What is the treatment that is being tested?
Pentoxifylline is made by a company called Sanofi. It is mainly used to treat people who have circulatory problems. But there is some evidence that it will also help your haemoglobin level.

What are the alternatives for treatment?
EPO is used to correct low haemoglobin levels in patients with renal failure. Unfortunately, not everyone responds to this hormone or needs a very high dose that can cause side effects and problems. You have been identified as someone who requires high doses of EPO. People like you may benefit if we find a treatment that makes you respond to EPO better.

What are the side effects of any treatment received when taking part?
Pentoxifylline is widely used for patients with circulation problems. The drug can increase bleeding. The use of Asprin and other anti-coagulants is safe with Pentoxifylline. If you are concerned about bleeding or have an increased tendency to bleed, please let us know.

Other side-effects are relatively rare but can include gastro-intestinal disturbance and headaches.

Some people can be allergic to Pentoxifylline or the "Dummy drug" which is composed of cellulose. If you are known to be allergic to either please let us know as you are not suitable for the study.

If you develop any side-effects or are concerned about the tablets/treatment, please contact us at any time. Details are provided below.

What are the other possible disadvantages and risks of taking part?
We think the risk of taking part is very low.

It is possible that Pentoxifylline is not effective at improving your Hb, but we will not be stopping or reducing you EPO.

If you have private medical insurance you should check with the company, before agreeing to take part in this study.

Harm to the unborn child
There is no evidence that Pentoxifylline is harmful to any unborn child. Nevertheless, we would advise that you take adequate precaution against getting pregnant during the study.

If you think you may be pregnant, you should tell us.
If you are trying to get pregnant, you should not take part in this study.

If you are of child bearing age, we will ask if you have missed your periods or if you think you might be pregnant before we do any "X-rays". If there is any doubt, we will do a pregnancy test before exposing you to "X-rays".

What are the possible benefits of taking part?
We cannot promise the study will help you but the information we get might help improve the treatment of people on dialysis.

You have been identified as someone who does not respond well to EPO. This makes you at risk of becoming anaemic and getting side effects from EPO. If you are randomized to Pentoxifylline, you may respond better to EPO. If you are randomized to the "dummy" tablet, you will not get any potential benefit from Pentoxifylline. However, the study team will keep a close eye on your results throughout the study and make sure your treatment is adjusted according to the dialysis unit's guidelines. This "study effect" has been seen in many different studies and can be quite significant.

What happens when the research study stops?

At the end of the study, you will stop your treatment. However, Pentoxifylline is a licenced drug and can be continued if you need it.

What if there is a problem? Who can I contact?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

If you have any complaints or have any questions, please contact:

Research Nurse: Mr. Wancheung Li (through Royal London Hospital switch board)
Consultant in charge: Tel:020 3594 2674 (Dr Fan) or 020 3594 2658 (Prof Yaqoob)

In emergencies, please contact the Renal Registrar on call: Tel: 020 7377 7000

Alternatively, you can contact:
Patient Advice and Liaison Service (PALS)
Telephone: 020 7943 1335, Minicom: 020 7943 1350
E-mail:

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. If you consent to take part in the research the people conducting the study will abide by the Data Protection Act 1988, and the rights you have under this Act.
All the information about your participation in this study will be kept confidential. The details are included in Part 2.'

This completes Part 1 of the Information Sheet.
If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What will happen if I don't want to carry on with the study?
If you chose to withdraw from the study any information that has been already collected will be processed as part of the study.

If you stop the treatment, we will ask if you are willing to have a 1-month "Off-Treatment" follow-up. This is important as we want to see what happens to your blood results and Hb levels after you come off the treatment. We shall also ask if you are willing to have the end of study "X-rays".

You are free to say "no" to the "off-treatment follow-up", the extra blood tests or to the "X-rays". However, you should be aware that audits are conducted on a regular basis as required by Good Clinical Governance. Results of blood tests that are taken as part of your regular medical care will continue to contribute towards our audit and reporting to Department of Health mandated registries.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (See contact details that are listed in Part 1 of this form). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

Harm:
In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against Barts Health NHS Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).'

NHS Indemnity does not offer no-fault compensation i.e. for non-negligent harm, and NHS bodies are unable to agree in advance to pay compensation for non-negligent harm. They are able to consider an ex-gratia payment in the case of a claim.

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. If you consent to take part in the research the people conducting the study will abide by the Data Protection Act 1988, and the rights you have under this Act.

Information including details of your treatment and details about any infection you may develop during the study will be collected and entered into a database. This will be coded and the investigators will keep the code. People that are not involved in the study will not have access to the code. The information will be kept for 1520 years and in accordance with the sponsor policy.

Involvement of the General Practitioner/Family doctor (GP)
Unless you object, we will inform your GP that you are taking part in this study and details about your treatment. We think this is important so they know what tablets you are taking.

What will happen to the results of the research study?
We hope the results of our study will be important to all doctors looking after patients undergoing HD. We will therefore publish the results and present the data at various meetings. However, at all times, your confidentiality will be protected. You will NOT be identified in any report/publication unless we ask you for specific permission.

Who is organising and funding the research?
Barts Health NHS Trust is organising and running this study.

Who has reviewed the study?
This study was given a favourable ethical opinion for conduct in the NHS by the East London and The City Research Ethics Committee. The detailed study has also been carefully considered by an independent internal research committee of the Renal Unit at BHT

You may wish to thank your participant for considering taking part or taking time to read this sheet.

Barts Health NHS
NHS Trust

Centre Number: : Patient Id::

CONSENT FORM

Title of Project: PEntoxifylline in Anaemia Resistant to erythropoietin (PEAR)

Name of Researcher: Prof M Yaqoob **Please initial box**

1. I confirm that I have read and understand the information sheet dated 29th Oct. 2013 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from Barts and The London or from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

6. I understand that blood will be taken during my treatment / investigation and will not be used for diagnostic purposes. I agree that this blood will be stored in a Research Tissue Bank for future research. ☐

_____	_____	_____
Name of Patient	Signature	Date
_____	_____	_____
Name of Person taking consent (if different from researcher)	Signature	Date
_____	_____	_____
Researcher	Signature	Date

15.4 Ethics committee and MHRA approval

NHS
Health Research Authority

NRES Committee London - City & East
Bristol Research Ethics Committee Centre
Whitefriars
Level 3, Block B
Lewins Mead
Bristol
BS1 2NT

Tel: 01173421386
Fax: 01173420445

05 March 2013

Professor Magdi Yaqoob
Barts and The London
The Royal London Hospital
Whitechapel
E1 1BB

Dear Professor Yaqoob,

Study title:	**A Single-Centre randomized placebo controlled, double blinded study of Pentoxifylline in End-Stage Renal Disease Patients with Erythropoeitin resistance**
REC reference:	**12/LO/1635**
EudraCT number:	**2011-006168-30**
Amendment number:	**AM01 Substantial Amendment 01 dated 04 Dec 2012**
Amendment date:	**22 February 2013**
IRAS project ID:	**103187**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Consent Form: Participant Consent Form	4	28 August 2012
Participant Information Sheet: Participant Information Sheet	4	28 August 2012
European Commission Notification of Substantial Amendment Form	AM01 Substantial Amendment 01 dated 04 Dec 2012	22 February 2013
Covering Letter		27 September 2012
Protocol	7-1	27 November 2012

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

12/LO/1635:	**Please quote this number on all correspondence**

Yours sincerely,

pp Dr Arthur T. Tucker
Chair

E-mail: nrescommittee.london-cityandeast@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mr Gerry Leonard, Barts Health NHS Trust*

NRES Committee London - City & East

Attendance at Sub-Committee of the REC meeting on 25 February 2013

Name	*Profession*	*Capacity*
Professor Atholl Johnston	Professor of Clinical Pharmacology	Expert
Dr Arthur T. Tucker	Principal Clinical Scientist & Honorary Reader, (REC Chairman)	Expert
Professor David Wingate	Gastroenterologist	Expert

Also in attendance:

Name	*Position (or reason for attending)*
Mr Rajat Khullar	Committee Coordinator

Safeguarding public health

MHRA

Prof S Fan
BARTS AND THE LONDON NHS TRUST
RENAL UNIT
THE ROYAL LONDON HOSPITAL
LONDON
E1 1BB
UNITED KINGDOM

25/03/2013

Dear Prof S Fan

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	19717/0231/001-0003
Eudract Number:	2011-006168-30
Product:	TRENTAL 400 TABLETS 400MG
Protocol number:	RLH_Pentoxifylline_Dec2011
Substantial Amendment Code Number:	Code Number: substantialAmendment_2
Version: 1	
Date: 2013/02/21	

NOTICE OF ACCEPTANCE OF AMENDMENT

I am writing to inform you that the Licensing Authority accepts the proposed amendment to your clinical trial authorisation (CTA), received on 08/03/2013.

This amendment may therefore be made.

You are reminded that where it is appropriate, the Ethics Committee should also be notified of amendments.

Yours sincerely,

Clinical Trials Unit
MHRA